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CONTENTS OF VOLUME 31, 1941

NO. 1. JANUARY

Abstracts of papers presented at the thirty-second Annual Meeting of The American Phytopathological Society, Philadelphia, Pa., Dec. 27–31, 1940 inclusive 1
Some problems in handling and interpreting plant disease data in complex factorial designs. W. H. Tharp, C. H. Wadleigh and H. D. Barker 26
Pathogenicity studies with isolates of Rhizoctonia solani obtained from potato and sugar beet. E. L. LeClerg 49
Brown-spot needle disease of pines. Frederick A. Wolf and W. J. Barbour 61
Preliminary investigations on dwarf bunt of wheat. C. S. Holton 74
Association of Stereum murrayi with heart rot and cankers of living hardwoods. Ross W. Davidson, W. A. Campbell and Rolland C. Lorenz 82
The Rhizoctonia foot-rot of annual stocks (Matthiola incana). A. W. Dimock 87
Phytopathological Notes 91
  The growth of Fusarium oxysporum cubense in the soil. Clifford H. Meredith
  Infection with pure cultures of Clitocybe tabescens. A. G. Plakidas
  Algal leaf and fruit spot of guava. Geo. D. Ruehle
  Conidial production in culture by Cercospora nicotianae. Stephen Diachun and W. D. Valleau
Book Reviews 98

NO. 2. FEBRUARY

Pathogenic and cultural variation among single-spore isolates from strains of the tomato-wilt Fusarium. Frederick L. Wellman and Dorothy J. Blaisdell 103
Further evidence for multiplication of the aster-yellows virus in the aster leaf hopper. L. M. Black 120
Development of secondary tumors and tumor strands in the crown gall of sunflowers. Armin C. Braun 135
Quantitative comparison of methods for sterilizing solutions of organic compounds used in culture media. Glen A. Greathouse and Neil E. Rigler 149
Fungi associated with diseases of cotton seedlings and bolls, with special consideration of Glomerella gossypii. R. Weindling, P. R. Miller and A. J. Ullstrup 158
Morphological and anatomical features of phyllody in varieties of tomatoes and beans. B. F. Dana 168
Effect of X-radiation on the germination of chlamydospores of Ustilago hordei. H. A. Rodenhiser and L. R. Maxwell 175
The shape and development of potato tubers, and their significance in the diagnosis of spindle tuber. J. G. Bald, D. O. Norris and B. T. Dickson 181
Eye spot of lemon grass. B. A. Bourne 186
Germination experiments with uredio- and teliospores of Tranzschelia pruni-spinosae discolor. John C. Dunegan and Clayton O. Smith 189
Bacterial leaf spot of Gardenia. W. H. Burkholder and P. P. Pirone 192
A new Ascochytella on Pentstemon from California. Anna E. Jenkins 194
Phytopathological Notes 197
  Longevity of teliospores of Puccinia graminis under laboratory conditions. T. Johnson
  Bacterial wilt of carnation. Leon K. Jones
Report of the Committee on Biological Abstracts and the Union of American Biological Societies. Howard P. Barss 199
Book Review 200

NO. 5. MARCH

Techniques for appraising air-borne populations of microorganism, pollen, and insects.
  The Committee of Apparatus in Aerobiology, National Research Council 201
Purple leaf spot of strawberry. A. G. Plakidas 225
Leaf-spot diseases of poplars caused by Septoria musiva and S. populicola. G. E. Thompson 241
A heritable lysis in germinating chlamydospores of Sphacelotheca sorghi. Thomas Laskaris 254
Thermal death point of fungi in relation to growing conditions. Lee Ling and Emerson H. Yu 264
Fungi associated with stain in chemically treated green lumber. A. F. Verrall 270

Comparative studies of sugar-beet and potato isolates of Rhizoctonia solani. E. L. LECLERG 274
Longevity of sclerotia of the stem-rot fungus Leptosphaeria salvinii. E. C. TULLIS AND E. M. CRALLEY 279
Epinasty of tomato, one of the earliest symptoms of Fusarium wilt. FREDERICK L. WELLMAN 281
Phytopathological Notes 284
  A technique for identifying the loose smuts of barley. V. F. TAPKE
  A copper-soap spray for control of tobacco downy mildew. RANDALL R. KINCAID

NO. 4. APRIL

Haven Metcalf. E. P. MEINECKE 289
Experiments with eradicant fungicides for combating apple scab. G. W. KEITT, C. N. CLAYTON AND M. H. LANGFORD 296
Host range of peach-mosaic virus in western Colorado. E. W. BODINE AND L. W. DURRELL 322
Variation in the germination of chlamydospores of Ustilago zeae. M. F. KERNKAMP AND M. A. PETTY 333
Studies on the nature of spindling sprout of potato. G. K. PARRIS AND WINSTON W. JONES 340
The black rot of Barbarea vulgaris. WALTER H. BURKHOLDER 347
Phytopathological Notes 349
  Rosy canker of London plane associated with illuminating gas injury. C. MAY, J. M. WALTER AND P. V. MOOK
  Resistance to floral-infecting loose smut (Ustilago nuda) in fall-sown barley varieties at Statesville, North Carolina. G. K. MIDDLETON AND W. H. CHAPMAN
  A new case of rosette mosaic on peach. E. M. HILDEBRAND
  Cherry yellows (physiological yellow leaf) in New York. E. M. HILDEBRAND AND W. D. MILLS
  Citrus viruses. HOWARD S. FAWCETT
  Powdery mildew of potato in Kentucky. W. D. VALLEAU
  Inoculation of cotton plants in sand culture with Phymatotrichum root rot. A. A. DUNLAP
  Olive knot induced on species of the Oleaceae by artificial inoculations. CLAYTON O. SMITH
Report of the thirty-second annual meeting of The American Phytopathological Society 362

NO. 5. MAY

Further experiments on the control by seed disinfection of root-rotting fungi in wheat. J. E. MACHACEK AND F. J. GREANEY 379
Purification and properties of alfalfa-mosaic virus protein. A. FRANK ROSS 394
The concentration of alfalfa-mosaic virus in tobacco plants at different periods of time after inoculation. A. FRANK ROSS 410
Studies on mosaic of cowpeas, vigna sinensis. D. M. MCLEAN 420
Brazilian bean varieties as plant indicators for the tobacco-mosaic virus. K. SILBERSCHMIDT AND M. KRAMER 430
Botrytis and Sclerotinia as potato tuber pathogens. GLEN B. RAMSEY 439
A photoelectric method and its use for determination of fungus growth rates. L. A. ADAIR AND ELIZABETH JANE MOORE 448
Field tests of the resistance of cotton to Phymatotrichum omnivorum. G. W. GOLDSMITH AND ELIZABETH JANE MOORE 452
Vein clearing, a transmissible disease of Prunus. S. M. ZELLER AND A. W. EVANS 463
The Boron-deficiency disease of gloxinia and its control. P. A. ARK AND C. M. TOMPKINS 467
Phytopathological Notes 469
  Incidence of Sclerotinia fructicola and S. laxa on sweet cherries in Oregon. A. W. EVANS AND C. E. OWENS
  Single-spored and Monosporous vs. . . . ? FREEMAN WEISS
Announcement 472

NO. 6. JUNE

James Franklin Collins. WALTER H. SNELL AND ALMA M. WATERMAN 475
Three species of Pythium with proliferous sporangia. CHARLES DRECHSLER 478
Two physiologic races of Helminthosporium maydis in the corn belt. ARNOLD J. ULLSTRUP 508

Experimental production of symptoms in so-called recovered ringspot tobacco plants and its bearing on acquired immunity. W. D. VALLEAU 522
The inheritance of resistance to powdery mildew (Erysiphe cichoracearum) in lettuce. THOMAS W. WHITAKER AND DEAN E. PRYOR 534
Tumors on elm and maple trees. NELLIE A. BROWN 541
A solution-culture infection method used in the study of Fusarium wilts. G. M. ARMSTRONG 549
The effect of leaf rust on the carotene content of white clover. J. T. SULLIVAN AND S. J. P. CHILTON 554
Inheritance of resistance to stem rust in crosses with Kenya varieties of Triticum vulgare Vill. I. A. WATSON 558
Phytopathological Notes 561
  Further notes on fig mosaic. IRA J. CONDIT AND W. T. HORNE
  The effect of sodium nitrate on Fusarium oxysporum cubense. CLIFFORD H. MEREDITH
  Myriogenospora on sugar cane in Louisiana. E. V. ABBOTT AND R. L. TIPPETT
  Preparation of inoculum with a mechanical liquefier. C. F. ANDRUS
  The mycorhizal fungi and mycorhizae of four coniferous plantations in the Rhine Valley. W. D. THOMAS, JR..
  Fluorescence of diseased potatoes. L. H. FLINT AND C. W. EDGERTON
  The use of methyl bromide as a means of detecting latent infections by Colletotrichum spp. G. K. PARRIS AND W. W. JONES
Announcements 572
  Pre-publication notice of a review of sexuality in the higher fungi by Professor A. H. Reginald Buller. FREEMAN WEISS
  Symposium papers available.
  Summer meeting of the north central States group of phytopathologists.

NO. 7. JULY

The behavior of certain viruses in plant roots. ROBERT W. FULTON 575
Fusarium wilt of the mimosa tree (Albizzia julibrissin). E. RICHARD TOOLE 599
Factors affecting infection in rye smut and subsequent development of the fungus in the the host. LEE LING 617
Parasitism of the root-knot nematode in leaves and stems. M. B. LINFORD 634
Transmission of plant viruses by dodder. FOLKE JOHNSON 649
Transmission of the Dutch elm disease pathogen by Scolytus multistriatus and the development of infection. F. G. PARKER, PHILIP A. READIO, LEON J. TYLER AND DONALD L. COLLINS 657
Stem blight of cotton caused by Alternaria macrospora. LEE LING AND JUHWA Y. YANG 664
Phytopathological Notes 672
  The relative susceptibility of different lots of oat varieties to smut. IAN W. TERVET
  Rhizoctonia root rot of sweet clover. W. J. CHEREWICK
  "Bends," a new disease of grasses and cereals. GEORGE W. FISCHER
Book Review 677

NO. 8. AUGUST

Chemical inactivation and the reactivation of a plant virus. JAMES JOHNSON 679
Actinomyces in potato tubers. B. F. LUTMAN 702
Brown rot on stone fruits in western Washington. GLENN A. HUBER AND KARL BAUR 718
Blister rust studies of three patches of red currants in New York. WALTER H. SNELL 732
Sporulation injury associated with downy-mildew infections. C. E. YARWOOD 741
Effect of root-knot nematode on tomato wilt. A. L. HARRISON AND P. A. YOUNG 749
Variations in Phytomonas vesicatoria. WALTER H. BURKHOLDER AND C. C. LI 753
Phytopathological Notes 756
  Classification of Hawaiian Commelina-mosaic virus. W. C. PRICE
  Comparison of susceptibility of the American elm and several exotic species to Ceratostomella ulmi. S. J. SMUCKER
  Isometric crystals produced by Pisum virus 2 and Phaseolus virus 2. FRANK P. MCWHORTER
  Variation in cultures of certain grass smuts. W. J. MARTIN AND M. F. KERNKAMP
  Storage and mounting of demonstration specimens. JOHN EHRLICH
  Evidence of resistance in alfalfa, red clover, and sweetclover to certain fungus parasites. F. R. JONES, J. L. ALLISON AND W. K. SMITH
Report of the 1941 annual meeting of the Southern Division of The American Phytopathological Society 767

NO. 9. SEPTEMBER

Some hyphomycetes parasitic on free-living terricolous nematodes. CHARLES DRECHSLER ... 773
Variation in culture of several isolates of Armillaria mellea from Western white pine. VINCENT L. BENTON AND JOHN EHRLICH ... 803
Some relations between mercuric chloride content, acid content, and fungicidal efficiency of certain solutions as used for potato-tuber disinfection. BERNIE E. PLUMMER, AND REINER BONDE ... 812
Soil treatments with volatile liquids for control of nematodes. B. G. CHITWOOD ... 818
The growth rate of several wood-inhabiting fungi. A. E. EDGECOMBE ... 825
The relation of the curculionid Anacentrinus deplanatus, to root rot and basal stem rot of barnyard grass, Echinochloa crusgalli. E. W. HANSON AND H. E. MILLIRON ... 832
Current-season development of virus symptoms in tulips. PHILIP BRIERLEY ... 838
Calcium deficiency as a factor in abnormal rooting of philodendrons. C. M. TUCKER AND P. R. BURKHOLDER ... 844
Stunt disease of strawberry. S. M. ZELLER AND L. E. WEAVER ... 849
Pathogenicity of aeciospores obtained by selfing and crossing known physiologic races of Melampsora lini. H. H. FLOR ... 852
Phytopathological Notes ... 855
- Reactions of Napier grass, Merker grass, and their crosses to Helminthosporium eye spot. G. K. PARRIS AND J. C. RIPPERTON
- Identity and distribution of three rusts of corn. GEORGE B. CUMMINS
- Control of damping-off of broadleaf seedlings. ERNEST WRIGHT

Report of the 1941 annual meeting of the Pacific Division of The American Phytopathlogical Society ... 859

NO. 10. OCTOBER

Hofrat Professor Dr. Gustav Köck. DOZENT DR. ING. HANS STEINER ... 867
Concentration and characterization of the emetic principle present in barley infected with Gibberella saubinetii. WILLIAM G. HOYMAN ... 871
Transmission of diamond canker of the French prune. RALPH E. SMITH ... 886
Cytospora canker of Italian cypress. GEORGE A. ZENTMYER ... 897
Host-parasite relationships in pink root of Allium cepa. II. The action of Phoma terrestris on Allium cepa and other hosts. W. A. KREUTZLER ... 907
The buckskin disease of cherry and other stone fruits. T. E. RAWLINS AND H. EARL THOMAS ... 916
Are ammonium salts toxic to the cotton root rot fungus? LESTER M. BLANK AND PAUL J. TALLEY ... 926
A Xylaria tuber rot of potato. GEO. D. RUEHLE ... 936
New facts about eastern snowmold. C. C. WERNHAM ... 940
Sclerotium rolfsii on cotton in Arizona. MANFRED GOTTLIEB AND J. G. BROWN ... 944
Phytopathological Notes ... 947
- Poinsettia scab caused by Sphaceloma. GEO. D. RUEHLE
- An apparently undescribed disease of the peanut (Arachis hypogaea). WILBERT A. JENKINS
- Fall-sown spring wheat susceptible to dwarf bunt. R. H. BAMBERG
- A satisfactory medium for germination of urediospores of Puccinia graminis tritici. WILLIAM Q. LOEGERING
- Spore germination of Selenophoma bromigena. ELLIS F. DARLEY
- The use of iodine in the control of potato ring rot and scab. P. A. ARK
- Chemical eradication of crown gall on almond trees. P. A. ARK
- Observations on the epidemiology of tobacco wildfire and blackfire. WILLIAM B. ALLINGTON

Book Review ... 960

NO. 11. NOVEMBER

William Williams Gilbert ... 961
Comparative physiology of crown gall, attenuated crown gall, Radiobacter, and hairy root bacteria. A. J. RIKER, MARY M. LYNEIS, AND S. B. LOCKE ... 964
Synthesis of tobacco mosaic virus protein in relation to leaf chromo-protein and cell metabolism. MARK W. WOODS AND H. G. DUBUY ... 978
Experimental consideration of the mold toxins of Gliocladium and Trichoderma. RICHARD WEINDLING ... 991
The line-pattern virosis of the genus Prunus. DONALD CATION ... 1004
Damping-off of longleaf pine. WILLIAM C. DAVIS ... 1011
Injury to tomatoes by lightning. OTIS C. WHIPPLE ... 1017

Effect of fertilizer materials and soil amendments on development of apothecia of Sclerotinia fructicola. CARL BAUR AND GLENN A. HUBER 1023
A leaf spot of peas (Pisum sp.) caused by Cercospora lathyrina. J. L. WEIMER 1031
Silver nitrate as a stain for use in studies of conduction of liquids in wood. JOHN W. JOHNSON 1035
The vapor pressure gradient above a transpiring leaf. VERNON L. FRAMPTON AND KARLA LONGREE 1040
Smut sori from ovarial and staminal tissues of certain grasses. E. D. HANSING AND C. L. LEFEBVRE 1043
Interstate cooperative experiments on field spraying of sour cherries. H. W. THURSTON, JR., C. F. TAYLOR, A. B. GROVES, AND H. J. MILLER 1047
The pathogenicity of paired haploid lines of Ustilago zeae versus the pathogenicity of numerous mixed haploids. M. F. KERNKAMP AND W. J. MARTIN 1051
Phytopathological Notes 1054
  A fusarium wilt of sweet william (Dianthus barbatus). W. C. SNYDER
  Willow blight in British Columbia. I. L. CONNERS, A. W. MCCALLUM, AND J. E. BIER
  A low-temperature basidiomycete causing early spring killing of grasses and legumes in Alberta. W. C. BROADFOOT AND M. W. CORMACK
  Virus antagonism, natural host resistance, and the acquired immunity concept with reference to plants. H. H. MCKINNEY

NO. 12. DECEMBER

A root disease of Jeffrey and ponderosa pine reproduction. ALVER J. OLSON 1063
The probability law in cotton seedling disease. K. STARR CHESTER 1078
A distinctive strain of tobacco-mosaic virus from Plantago. FRANCIS O. HOLMES 1089
The reaction of cotton varieties to Fusarium wilt and root-knot nematode. A. L. SMITH 1099
The spore-germination method of evaluating fungicides. P. D. PETERSON 1108
Changes in nitrogen and virus content of detached tobacco leaves in darkness. WILLIAM N. TAKAHASHI 1117
Relation of Ceratostomella radicicola to rhizosis of the date palm. DONALD E. BLISS 1123
Verticillium wilt of strawflower. C. M. TOMPKINS AND P. A. ARK 1130
Saltants from a monosporic culture of Verticillium albo-atrum. JOHN T. PRESLEY 1135
Phytopathological Note 1140
  Resistance to the common mosaic disease of tobacco. E. E. CLAYTON AND H. H. MCKINNEY
Index for Volume 31 i

INDEX FOR VOLUME 31

New species in **blackface type**
Joint authorship indicated by pages in "( )"
Species index of bacteria included
AUTHOR AND SUBJECT INDEX

Prepared by Frederick V. Rand, Office of Experiment Stations, U. S. Department of Agriculture.

- AAMODT, O. S., 1
- ABBOTT, E. V., 564
- Acer spp., Phomopsis tumor of, 545
  - Phytophthora bleeding canker, 863
    - control by tree injection, 12
  - Stereum murrayi on, 82
- Aceratagallia sanguinolenta, as vector of potato yellow dwarf, hereditary variation in, 3
- ACREE, RUBY J., 1
- Acrobeloides bütschlii, Cephalosporium balanoides parasitizing, 786
- Acrostalagmus **bactrosporus** n. sp., nematodes parasitized by, 782
  - **obovatus** n. sp., nematodes parasitized by, 784
- Actinomyces scabies, culture media for, 714
  - on potato, control by iodine, 954
    - cyto- and histopathology, 702
    - pit-scab due to, insect and saprophyte roles in, 9
- ADAIR, L. A., 448
- ADAMS, M., (16)
- Aerobacter aerogenes, virus inactivation by growth products of, 690
- Aerobiology, techniques, 201
- aerogenes (Aerobacter), 960
- AFANASIEV, M. M., 1
- Age, curly top resistance of sugar beet vs. host-, 861
- Agglutination, micro-, of Ceratostomella spores, 15
- Agrostis palustris, fungus disease (new), Curvularia or Helminthosporium causing, 24
- Air, -centrifuge, 204, 205, 213
  - -currents, Phytomonas malvacearum disseminated by, 4
  - dissemination of microorganisms (etc.) by, appraisal technique, 201
  - sanitary analysis, 203
- Albizzia spp., Fusarium wilt, exp. infection, 608
  - julibrissin, Fusarium wilt, and cross inoculations, 1
    - distribution and general study, 599
- Alcaligenes radiobacter (see Bacillus radiobacter)
- Aldehydes, losses in sterilization, 153
- Alfalfa, killing by basidiomycete, 1058
  - mosaic, symptoms, as influenced by virus concentration, 418
    - virus, activity measurement, local lesion method, accuracy, 21
      - concentration in tobacco at intervals after inoculation, 410
      - extraction technique, 411
      - inactivation *in vivo*, 415, 419
      - purification and properties of protein of, 394
  - resistance to fungus parasites, 765
  - Rhizoctonia root rot, 674
- Aliphatic acids, losses in sterilization, 153
- Alkaloid, emetic principle of scabby barley as (?), 871
- ALLINGTON, WILLIAM B., 957
- ALLISON, J. L., (765)
- Allium spp. (see also Onion)
    - Phoma terrestris on, host-parasite relations, 907
    - smut resistance in, 9
- Almond, buckskin disease, symptoms, 920
  - Chalaropsis stem infection (exp.), 859
  - crown gall on, chemical eradication, 956
  - peach mosaic on, 323
  - peach ring rot grafted into, 860
- Alternaria spp., on carnation cuttings, as influenced by growth substances vs. potassium permanganate, 14
    - in chemically treated lumber stain, 272
    - cotton boll and seedling infection, 160, 769
    - on tomato, sprays for, timing and dosage, 11
  - macrospora, cotton stem blight due to, general study, 664
  - solani, on tomato, copper sprays tested for, 25
- Aluminum nitrate, effect on copper fungicide deposition on leaves, 860
- Alyssum spp., Rhizoctonia foot rot (exp.), 89
- Amanita muscaria, mycorhizal, on conifers, 568
- Amaranthus caudatus, tobacco ring spot inoculations, 580
- Ambrosia beetles, as vectors of Fusarium perniciosum, 611
- Ambrosia trifida, Acrostalagmus obovatus parasitizing, 784
- American Phytopathological Society, annual meeting report (1940), 362
  - Pacific Division, report of meeting (1941), 859
  - Southern Division, report of meeting (1941), 767
- Amides, losses in sterilization, 153
- Amines, losses in sterilization, 153
- Ammonium salts, toxicity to Phymatotrichum omnivorum, mechanism, 926
- Anacentrinus deplanatus, barnyard grass root rot and basal stem rot relations to, 832

    description, 834
Anaheim grapevine disease, development and spread, 862
Andropogon furcatus, Sorosporium smut sori on ovarial and staminal tissues, diagnostic value, 1043
    Sphacelotheca smut sori on ovarial tissues, diagnostic value, 1043
Andrus, C. F., 566
Angiopsora zeae, on corn, and distribution, 856
Anguillulina pratensis, on cotton, distribution in regional wilt plats, 771
angulatum (Bacterium), 957
Annual bluegrass (see Poa annua)
Annelids, potato pit-scab role, 9
Annulus tabaci, symptoms induced in ''recovered'' tobacco, 23
Antagonisms, microbiological, by mold-toxins, 991
    virus inactivation by, 690, 864
  virus-, 1059
Anthracnose(s), camellia Glomerella, 6
  celery Marssonia, 17
  Colletotrichum-induced, diagnosis by methyl bromide, 570
  cotton-, boll rot association of organisms of angular leaf spot and, 24
Antiseptic dressings, for wounds, 24
Aonodiella aurantii, on lemon, Bacillus ''C'' destroying, 864
Aphanomyces sp., canning pea root rot due to, control by ''Spergon'' seed protectant, 20
Aphids, extracts of cabbage leaves and, effect on oats coleoptile test, 15
  fungi parasitizing, 10
  as vectors of: cowpea mosaic, 423
    cucurbit viruses, 8
    strawberry stunt, 850
Aphis gossypii, as vector of cowpea mosaic, 423
Apothecia, factors influencing development in Sclerotinia, 1023
Apple, scab, epidemiology, 297
    eradicant fungicides for, 296
    ground sprays for, 13
    pathogenicity of fungus, genetic relations, 1142
    saltation in fungus, nature, 1142
    spray coverage index vs. inoculum potential, measurement technique and relations, 7
    Stereum murrayi on, 82
Apricot, buckskin disease on, 920
  peach mosaic on, 323
  Sclerotinia spp. on, 719
Ark, P. A., (14), 467, 954, 956, (1130)
Armillaria mellea, growth rate, comparisons, 826
    infection factors, 859
    inoculation methods, 859
    root rot due to, temp. relations, 3
    from western white pine, variations in culture, 803
Armstrong, G. M., 1, 549
Aromatic acids, losses in sterilization, 153
Ascochyta majalis, lily of the valley infected by, and distribution, 13
Ascochytella **pentstemonii** n. sp., pentstemon stem spot caused by, 195
Aspergillus spp., on cotton, 160
  niger, virus inactivation by growth products of, 690
Aster laevis, tobacco-ring-spot inoculations, 580
Aster yellows, heat therapy, 14
  transmission through dodder, 13
  virus, incubation and multiplication in vector, 126
    mechanical transmission between leaf hoppers, 124
    transmission through dodder, 651
Autoclave, in sterilizing organic compounds used in culture solutions, 152
Auxins (see Growth substances)
Avena coleoptile test, as influenced by extracts of cabbage leaves and aphids thereon, 15

Bacillus [=Alcaligenes] radiobacter, comparative physiology, 964
  ''C,'' red scale on lemon destroyed by, 864
Bacteria, air-borne populations, appraisal techniques, 201
  on barnyard grass, weevil relations, 832
  giant cactus necrosis caused by new, 15
  leaf invasion by, watersoaking relation, 6
  plant disease-, book review, 100
Bacterial spot, tomato-, 25
Bactericides, clove oil, for crown gall, 956
  Elgetol, for crown gall, 956
  iodine, for crown gall, 956
    for potato ring rot and scab, 954
  sodium dinitro-cresol, for crown gall, 956
Bacteriophagus n. gen. (viruses), 23
Bacterium (see also Phytomonas)
  angulatum, epidemiology of infection, light vs. watersoaking in, 957
  malvacearum, cotton boll rot association of Glomerella gossypii and, 24
  phaseoli, bean mosaic virus associated, and nutrient relations, 10
    dissociation, 10
  sepedonicum, on potato, diagnosis by fluorescence nonspecific, 569
  solanacearum, on tobacco, control by crop rotations, 20
  tabacum, epidemiology of infection, light vs. watersoaking in, 957
    on tobacco, mechanism of invasion, 6
  tumefaciens, invading overgrowths caused by, 15
  vesicatorium, on tomato, copper sprays tested on, 25
Baker, Kenneth F., 859
Bald, J. G., 181
Bamberg, R. H., 951
Banana, Panama disease, Fusarium of, growth in soil, 91
    sodium nitrate effects on, 564
Banfield, W. M., 2
Barbarea vulgaris, bacterial black rot (new), 347
barbareae (Phytomonas) n. sp., 348
barbareae (Zanthomonas) n. sp., 348
Barbour, W. J., (61)

Bark beetle(s), European elm-, as vector of Dutch elm disease, 657
  fungi parasitizing, 10
BARKER, H. D., (26)
Barley, covered smut, chlamydospore germination, X-ray effects, 175
  fall-sown, floral-infecting loose smut resistance in vars., 351
  Gibberella saubinetii infection, emetic principle in, concentration and characterization, 871
  loose smuts, differentiation and relationships, 22
    identification technique, 284
    seed treatment by Oklahoma hot-water seed treater, custom operation, 5
  Phoma terrestris infecting (exp.), 913
BARRETT, J. T., 2
Barss, Howard P., 98, 199
Basidiomycete, killing of grasses and legumes by low-temperature-, 1058
Basisporium gallarum, on corn, growth in cobs, factors influencing, 21
BAUR, KARL, 718, 1023
BEACH, W. S., 2
BEALE, HELEN PURDY, 2
Bean, Brazilian vars. as tobacco mosaic virus indicators, 430
  leaf variegation in, inheritance, 26
  Lima-, cowpea mosaic infection (exp.), 427
  mosaic virus, Bacterium phaseoli associated, and nutrient relations, 10
    behavior in roots vs. aerial parts of exp. hosts, 577
    inoculations, 579
  phyllody in vars., anatomy and morphology, 168
  Rhizoctonia isolates from potato vs. sugar beet on, 277
  rust, resistance to physiologic races, 26
  snap-, black root, pathology, 767
    Colletotrichum latant infection, diagnosis by methyl bromide, 570
    necrosis of tissues in, 767
Beauveria spp., insects parasitized by, 10
Beech (see Fagus)
Beet, garden-, boron deficiency in, 23
Beetles, ambrosia-, as vectors of Fusarium perniciosum, 611
  bark-, fungi parasitizing, 10
"Bends," grass and cereal disease (new), cause unknown, 674
Benne (see Sesamum indicum)
BENNETT, C. C., (1)
BENTON, VINCENT L., 803
BERCH, HENRY, (19)
Betula spp., Stereum murrayi on, 82
BIER, J. E., (1056)
Biographies and necrologies, Butler, Ormond Rouke, 374
  Collins, James Franklin, 475
  Gilbert, William Williams, 375, 961
  Köck, Gustav, 867
  Metcalf, Haven, 289, 376
Biological Abstracts, report of committee on, 199
Biological control, of insects by fungi, 10
  of mealy bugs by fungus parasite, 5
Biometry, cotton seedling disease study by, 1078
  of disease data in factorial designs, 26
Biovirales new order (viruses), 23
Birch (see Betula)
BLACK, L. M., 3, 98, 120
Black root, of snap beans, pathology, 767
Black spot, beet-, as boron deficiency, 23
Blackfire, tobacco bacterial, 957
BLAISDELL, DOROTHY J., (103)
BLANK, LESTER M., 926
Blight, benne Cercospora, 770
  carnation Alternaria, 14
  cotton Alternaria stem-, 664
  pecan, Elsinoë nursery-, 768
  potato Phytophthora, 17
  tomato Alternaria early-, 25
  willow-, Physalospora vs. Fusicladium as cause in Canada, 1056
BLISS, DONALD E., 3, 859, 1123
Blister rust, white pine- (see Cronartium ribicola)
BLODGETT, EARLE C., 859
BLODGETT, F. M., 3
Bluejoint turkeyfoot grass (see Andropogon furcatus)
Blue-stain, fungi of, ascospore development and morphology, 19
Blue-stem, potato-, varietal reactions to, 5
BODINE, E. W., 4, 322
Boletinus porosus, mycorhizal, on conifers, 568
Boletus spp., mycorhizal, on conifers, 568
Boll rot, cotton-, anthracnose and angular leaf-spot organisms associated in, 24
BONDE, REINER, (812)
Borax, beet black spot controlled by, 23
Bordeaux mixture (see under Fungicides)
Borer, California flatheaded, larval galleries, effect on liquid conduction in wood, determination, 1035
BORLAUG, N. E., 4
Boron deficiency, in beet, 23
  in cotton, symptoms, 771
  in gloxinia, 467
  in slipperwort, 469
  and excess, in citrus, 861
Botany, textbook, review, 677
Botrytis spp., in damping-off of longleaf pine, 1012
    potato tuber pathogen, 440
BOURNE, B. A., 186
BRAUN, ARMIN C., 135
Breeding (see also Genetics; Inheritance)
  bean, for rust resistance, 26
  cotton, for resistance to Fusarium wilt and root knot, 1099
  grasses, for disease resistance, 1
  rice, for Cercospora leaf spot resistance, 19
  tobacco, for mosaic resistance, 1140
Bremia spp., sulphur fungicide effects on, 865
BRIERLEY, PHILIP, 838
Broad ring spot, tobacco, virus, behavior in roots vs. aerial parts of exp. hosts, 577

Broadfoot, W. C., 1058
Broccoli, invading overgrowths in, 15
Bromus inermis, Sclenophoma leaf spot, spore germination, 953
Brown, A. M., (4)
Brown, J. G., 4, (15), (944)
Brown, Nellie A., 541
Brown rot, stone-fruit Sclerotinia, 9, 1023
Brown spot needle disease, pine Systremma, 61
Bryophyllum sp., crown gall on, temp. relations, 976
Bryovirus n. gen. (viruses), 23
Buckskin disease, of stone fruit trees, distribution, pathology, and virus strains, 916
  reactions of different hosts to virus strains, 864
Bulbs, rodent damage to, control, 23
Buller, A. H. Reginald, 4
Bunonema spp., Harposporium oxycoracum parasitizing, 797
  Nematoctonus tylosporus parasitizing, 779
Bunt, wheat-, Tilletia tritici dwarf race, 74, 951
Burke, O. D., 5
Burkholder, P. R., (844)
Burkholder, Walter H., 192, 347, 753
Bushy stunt, tomato-, transmission through dodder, 13, 652
Butler, Ormond Rourke, necrology, 374
Butt diseases, pine-, 25

"C" (Bacillus), 864
Cabbage, clubroot, control by mercurials, 18
  extracts of leaves and aphids thereon, effect on oats coleoptile test, 15
  Rhizoctonia foot rot (exp.), 89
  Rhizoctonia from potato vs. sugar beet on, 277
  tipburn, potash effects on, 18
Cactus, giant-, bacterial necrosis (new), 15
Cadophora spp., in chemically treated lumber stain, 272
Calceolaria herbeohybrida, boron deficiency disease of, 469
Calcium arsenate, compatibility of "inert" ingredients of fungicidal and insecticidal, 8
Calcium cyanamid, effect on apothecial development in Sclerotinia, 1023
Calcium deficiency, in cotton, symptoms, 771
  effect on rooting of Philodendron cuttings, 844
Calendula officinalis, tobacco ring-spot inoculations, 580
California grapevine disease, spread and development, 862
Calorific value, of decayed cordwood, 22
Camellia japonica, Glomerella anthracnose, 6
Campbell, W. A., (6), (82)
campestris (Phytomonas), 347
Canada streak, potato-, virus movement and route, 7
Canker(s), cypress Cytospora, 896
  French prune diamond-, virus-induced (?), 886
  hardwood-, Stereum murrayi association, 82
  London plane tree rosy-, illuminating-gas injury associated, 349
  peach Fusicoccum, 10
  Phytophthora cactorum-induced, in hardwoods, 12
  sycamore Ceratostomella, 23
Cantaloupe, downy mildew, control by dust fungicides, 769
  as influenced by fungicidal dusts, 769
  Phoma terrestris infecting (exp.), 914
  powdery mildew, as influenced by thiamin, 863
    resistance of host strains to races of, 863
Cantharellus cibarius, mycorhizal, on conifers, 568
Capitophorus fragaefolii, vector of strawberry stunt, 850
Capsicum frutescens, cucumber-mosaic inoculations, 579
Carnations, cuttings, diseases and rooting as influenced by growth substances vs. potassium permanganate, 14
Carnegiea gigantea, bacterial necrosis (new), 15
Caroselli, Nestor E., (12)
Carotene, in white clover leaf, as influenced by: rust infection, 554
    sulphur treatment, 556
Carrot, Phoma terrestris infecting (exp.), 913
Cassia tora, Fusarium wilt, and cross inoculations, 1
Cation, Donald, 1004
Cauliflower, bacterial peppery spot, notes, 347
  Phoma terrestris infecting (exp.), 914
Celery, Cercospora greenish black rot, 17
  tobacco mosaic inoculations, 579
Cells, micrugical method applied to, 11
Centrifuge, air-, 204, 205, 213
Cephaleurus virescens, guava leaf and fruit spot due to, 95
Cephalosporium acremonium, fig leaf spot due to (?), 771
  balanoides n. sp., nematodes parasitized by, 786
Ceratostomella spp., in chemically treated lumber stain, 272
    sycamore canker due to, transmission and control, 23
  ips, ascospore development and morphology, 19
  montium, ascospore development and morphology, 19
  radicicola, date palm rhizosis association, 1123
  ulmi, in bark beetle-infested elm wood, 19
    on elm, pathogenesis, 660
      species reactions to, 21
      susceptibilities of species compared, 758
      vascular invasion vs. vessel length, 2

    - microagglutination of spores, 15
    - on Planera aquatica, 21
    - transmission, by European elm-bark beetle, 657
- Cercospora sp., on peanut, control by sulphur dust, 16
  - brachypus, grape (muscadine) leaf spot induced by, 767
    - perfect stage, 767
  - cari, celery greenish black rot caused by, 17
  - lathyrina, pea leaf spot caused by, 1031
  - nicotianae, conidial production in culture, 97
  - oryzae, on rice, linkage in resistance factors, 19
  - sesami, on benne, control by hot water seed treatment, 770
- Cereals, "bends" disease of (new), cause unknown, 674
  - diseases of northern U.S.A., book review, 200
  - root rot and basal stem rot, weevil relations, 837
  - seed treatment by Oklahoma hot-water seed treater, custom operation, 5
- Chaetomium spp., on cotton, 160
- Chalaropsis thielavioides, rose bud-union failures due to, 859
- Chan, Shuk Yee, (2)
- Chapman, W. H., (351)
- Charles, V. K., 5
- Chemicals, virus inactivation and reactivation by, mechanism, 679
- Cherewick, W. J., 673
- Cherry, black- (see Prunus serotina)
  - Coccomyces leaf spot, control by copper fungicides, 22
  - mahaleb-, buckskin disease, symptoms, 919
    - line-pattern disease, 1007
  - peach mosaic on, 323
  - peach ring rot grafted into, 860
  - Sclerotinia spp. on, 720
  - sour-, buckskin disease, symptoms, 919
    - Coccomyces leaf spot, field spray tests (interstate cooperative), 1047
    - fungicidal effects on yields and quality, 16
    - yellows ("physiological yellow leaf"), history, distribution, and virus etiology, 355
  - sweet-, buckskin disease, reaction of peach vs., 864
      - symptoms, 917
    - Chalaropsis stem infection (exp.), 859
    - Sclerotinia spp. incidence in Ore. on, 469
  - vein clearing virosis (new), 464
- Chester, K. Starr, 5, 1078
- Chicory, invading overgrowths in, 15
- Childs, James F. L., 860
- Chilton, S. J. P., (554)
- Chitwood, B. G., 818
- Chive, smut resistance, 9
- Chlamydospores, Sphacelotheca, heritable lysis of germinating, 254
  - Ustilago, X-ray effects on germination, 175
- Chlorogenus spp., transmission through dodder, 649
  - callistephi var. vulgaris, mechanical transmission and behavior in vector, 120
- Chloropicrin, nematode control by soil treatment with, 818
- Chlorosis, citrus vein-, 860
  - in pin oaks, control by tree injections vs. soil treatments, 18
- Chokecherry, western- (see Prunus demissa)
- Christensen, J. J., (4), 200
- Chromoprotein, leaf-, relation to synthesis of virus protein, 978
  - quantitative determination technique, 980
- Ciferri, Raffaele, 960
- Cirsium arvense, Puccinia suaveolens on, systemic mycelia from urediospores, 4
- Citrivir, as genus name for citrus viruses, 356
  - **italicum** n. sp., citrus mottling virus, 357
  - **psorosis** n. sp., citrus psorosis virus, 357
    - var. **anulatum** n. var., citrus psorosis virus-B, 357
    - var. **vulgare** n. var., citrus psorosis virus-A, 357
      - heat resistance, 861
- Citrus, Armillaria root infection, factors in, 859
    - temp. relations, 3
  - heat resistance of tissues, 861
  - nutrient deficiencies and excesses in, symptoms, 860
  - psorosis, diamond canker of French prune compared, 895
    - heat resistance of A- virus, 861
  - viruses, nomenclature system suggested, 356
- Cladosporium spp., in chemically treated lumber stain, 272
- Clayton, C. N., (13), (14), (296)
- Clayton, E. E., 6, 1140
- Clitocybe tabescens, pear tree infection (exp.), 93
    - pure culture inoculation method, 93
    - tung tree root rot associated, 93
- Clove oil, crown gall on almonds controlled by, 956
- Clover, alsike- (see Trifolium hybridum)
  - red- (see Trifolium pratense)
  - white Dutch-, basidiomycete killing, 1058
- Clubroot, cabbage-, 18
- Coccomyces hiemalis, on cherry, control by copper fungicides, 22
    - on sour cherry, field spray tests (interstate cooperative), 1047
- Cochran, L. C., 860, (861)
- Cole, John R., (768)
- Collembola sp., potato pit-scab role, 9
- Colletotrichum spp., diagnosis of latent infections by methyl bromide, 570
    - papaya latent infection, diagnosis, 570
  - destructivum, sporulation, loss of, 5
  - falcatum, on sugar cane, spore load vs. incidence of infection, 767
    - virulence, origin of loss in culture, 767
  - glycines, thermal death point, 264
  - gossypii, thermal death point, 264
  - lindemuthianum, on snap beans, diagno-

    sis of latent infection by methyl bromide, 570
  nicotianae, thermal death point, growing conditions vs., 264
Collins, Donald L., (19), (657)
Collins, James Franklin, biographical sketch and portrait, 475
Collybia velutipes, growth rate, comparisons, 826
Commelina nudiflora, mosaic, virus hosts and classification, 756
Compost, ''ratsnest,'' boron deficient in, 468
Condit, Ira A., 561
Conidiobolus spp., insects parasitized by, 10
Conifers, mycorhizas of, in Germany, 567
Conners, I. L., 1056
Cooley, J. S., 6
Copper deficiency in citrus, 860
Copper fungicides (see under Fungicides)
Cordwood, decayed, chemical composition and calorific value, 22
Cormack, M. W., (1058)
Corn, Basisporium cob rot, factors influencing infection, 21
  dent, bacterial wilt, susceptibility of inbreds to, and age relations, 7
  Diplodia zeae, spread in pith of hybrid vs. inbred lines, 20
  Gibberella saubinetii spread in pith of hybrid vs. inbred lines, 20
  Helminthosporium maydis infection, distribution and general study, 508
  Phoma terrestris infecting (exp.), 913
  rusts, identity and distribution, 856
  Sorosporium smut, on male inflorescence, 1046
  Ustilago smut, chlamydospore germination variations, 333
    on male inflorescence, 1046
    pathogenicity of paired haploid lines vs. numerous mixed haploids, 1051
Corticium vagum, potato tuber disinfection for, 812
Cortinarius spp., mycorhizal, on conifers, 568
Cotton, Alternaria stem blight, general study, 664
  angular leaf spot, wind dissemination, 4
  boll rot, anthracnose and angular leaf spot organisms associated in, 24
  fungi on seeds and bolls (1938–40), 769
  Fusarium wilt, and cross inoculations, 1
    pathogenicity of isolates to vars., 770
    regional varietal studies, 770
      nematode distribution in, 771
      and phosphorus relations, 771
    solution-culture methods for inoculation, 549
  mineral deficiency symptoms in greenhouse, 771
  Phymatotrichum root rot, inoculations in sand culture, technique, 358
    resistance to, field selection tests for, 452
  root knot resistance, 771
  seed, fuzzy vs. mechanically delinted, 769
    germination of treated, as influenced by storage, 768
  seed treatments, by organic mercurials, mode of action, 768
    for seedling diseases, 1078
    tests in 1940, 769
  seed-borne infections, hazard of, 1078
  seedling and boll infections, fungi associated, 158
  seedling diseases, control by seed treatments, 1078
    probability law in, 1078
  southern sclerotial rot in Arizona, 944
  varietal reactions to Fusarium wilt and root knot, 1099
Couch, J. N., (5)
Cowpea, Fusarium wilt, and cross inoculations, 1
  mosaic of, distribution, hosts, transmission and general study, 420
    virus properties, 424
  root knot parasitism in leaves and stems, 637
Cralley, E. M., (279)
Crinkle, strawberry-, stunt infection plus, 850
Cronartium spp., flexuous hyphae in, 4
  occidentale, differentiation, pH method as influenced by temp., 1
  ribicola, differentiation, pH method as influenced by temp., 1
    on red currant, relative susceptibility and potential inoculum volume, 732
Crop plants, rotation (see Rotation)
Crop yields, missing plants vs., determination method, 3
Crown diseases, pine-, 25
Crown gall (see also Overgrowths; Tumors)
  on almond, 956
  comparative physiology of bacteria of, 964
  growth substance relations, 19
  invading overgrowths in, 15
  sunflower-, secondary tumor and strand development in, 135
  on tomato, 11
Crucifers, bacterial black rot, notes, 347
Cucumber, downy mildew, copper fungicides as influenced by Al in control of, 860
  mosaic, virus, relation to commelina mosaic virus, 756
    virus-1, behavior in roots vs. aerial parts of exp. hosts, 577
  Phoma terrestris infecting (exp.), 913
  virus inoculations, 579, 580
Cucurbita moschata, curly top reactions of, 860
Cucurbits, mosaics, transmission via dodder, 13, 652
  viruses of, insect transmission, 8
Cultures, single-spore terminology, 471
Cummins, George B., 856
Cunninghamella **meineckella** n. sp., pine root disease due to, 1076

Cupressus spp., Cytospora canker, general study, 896
  sempervirens var. stricta, Cytospora canker, general study, 896
Curly top, Cucurbita reactions to, 860
  dodder histological relation to hosts infected by, 861
  sugar beet-, resistance vs. host age, 861
    virus, leaf hopper vector relations to strains, 8
      strain combinations, 8
      transmission via dodder, 13, 652
  on tomato, plant populations in relation to, 864
Currant, red (see Ribes vulgare)
Curves, toxicity-, of compounds as rated by different fungi, 16
Curvularia sp., grass disease caused by (?), 24
Cuscuta californica, histological relation to healthy vs. curly-top-infected sugar beet and tobacco, 861
  campestris, viruses transmitted via, 13, 649
Cushaw, curly top reactions of, 860
Cuttings, carnation-, as influenced by growth substances vs. K-permanganate, 14
  Philodendron, abnormal rooting vs. Ca-deficiency, 844
Cymadothea trifolii, red clover resistance to, 766
Cymbopogon citratus, Helminthosporium eye spot, 186
Cynoglossum amabile, virus inoculations, 579, 580
Cypress, Italian (see Cupressus sempervirens var. stricta)
Cytospora cenisia f. **littoralis** n. f., cypress canker due to, history, distribution and general study, 902

Dahlia, invading overgrowths in, 15
Daines, R. H., (10)
Dallas grass (see Paspalum dilatatum)
Damping-off, of broadleaf seedlings, forms and rotation effects, 857
  cotton-, 1078
    fungi associated, 158, 679
  as influenced by nitrates, and control by glucose, 858
  of longleaf pine, 1011
  rhubarb Pythium, 863
Dana, B. F., 168, 860
Darley, Ellis F., 953
Datura stramonium, virus inoculations, 580
Davidson, Ross W., 6, 82
Davis, B. H., 6
Davis, William C., 1011
Decays (see Rots)
Decker, Phares, (3)
Deficiency diseases (see also Physiological diseases)
  boron-, in beet, 23
    in gloxinia, 467
    in slipperwort, 469
  citrus-, 860
  cotton mineral-, 771
  peanut mineral-(?), 948
  Philodendron cuttings abnormally rooted, Ca-deficiency role, 844
Defoliation disease, of holly cuttings, Rhizoctonia-induced, 6
Demonstration specimens, storage and mounting technique, 763
Dermacentroxenus, as virus genus, 23
Diabrotica soror, as vector of cucurbit viruses, 8
  trivittata, as vector of cucurbit viruses, 8
Diachun, Stephen, 97
Diagnosis, of Colletotrichum latent infections, by methyl bromide, 570
Diamond canker, of French prune, virus-induced (?), 886
Dianthus barbatus, Fusarium wilt (new) of, 1054
Dickson, B. T., (181)
Dickson, James G., 200
Diffusion, forced, vapor pressure gradient above transpiring leaf following same general equation as for, 1040
Dimock, A. W., 87
Dimond, Albert E., 7, (12)
Diplodia spp., in chemically treated lumber stain, 272
    on cotton, 160
  gossypina, cotton boll and seedling infection, 769
  zeae, on corn, spread in pith, 20
Diseases (plant), book review, 960
  of cereal and forage crops in northern U.S.A., 200
  data in complex factorial designs, handling and interpreting, 26
  epidemiology, 215
  grass-, control by selection and hybridization, 1
  insect transmission, book review, 98
  in oak-hickory stands in Iowa, 99
  parasitic, book review, 100
  phanerogamic, book review, 960
  root-infecting types, solution-culture inoculation methods, 549
  vegetable crop-, book review, 98
Dissociation, microbic, in Bacterium phaseoli, 10
Distribution of: Angiopsora zeae, 856
  Ascochyta majalis, 13
  cowpea mosaic, 420
  Cytospora cenisia f. littoralis, 896
  elm mosaic, 22
  Fusarium sp. on lupines, 771
  Fusarium perniciosa, 599, 615
  Fusicladium saliciperdum, 1056
  Fusicoccum amygdali, 10
  line-pattern virosis of stone fruits, 1004
  Mycosphaerella louisianae, 228
  Mycosphaerella populicola, 251
  Mycosphaerella populorum, 242
  Myriogenospora sp. on sugar cane, 564
  Physalospora miyabeana, 1056
  Puccinia polysora, 856
  Sclerospora macrospora, 768
  Sclerotinia spp., 718
Ditylenchus dipsaci, soil treatments with volatile liquids for, 818

Dodder, histological relation to healthy vs. curly-top-infected sugar beet and tobacco, 861
Dodder, viruses transmitted via, 13, 649
Dolichos lablab, tobacco ring spot inoculations, 580
Dorrell, William W., 7
Drechsler, Charles, 478, 773
Dressings, antiseptic, for wounds, 24
DuBuy, H. G., (25), (978)
Duggar, B. M., (19)
Dunegan, John C., 189
Dunlap, A. A., 358
Durrell, L. W., (4), (322)
Dutch elm disease (See Ceratostomella ulmi)
Dykstra, T. P., 7

Echinochloa crusgalli, root rot and basal stem rot, weevil relations to, 832
Edgecombe, A. E., 825
Edgerton, C. W., (569)
Egg yolk, downy mildew conidial stimulation by, 865
Eggers, Virginia, (15)
Eggplant, Phoma terrestris infection (exp.), 914
  Verticillium albo-atrum infection (exp.), 1134
Ehrlich, John, 763, (803)
Elgetol, crown gall on almonds controlled by, 956
  as eradicant spray for apple scab, 13
Elliott, Charlotte, 7
Elm (see Ulmus)
  dwarf Asiatic- (see Ulmus pumila)
Elsinoë randii, pecan nursery blight caused by, fungicidal control, 768
Emetic principle, of scabby barley, concentration and characterization, 871
Emilia sonchifolia, root knot parasitism in leaves and stems, 636
Enations, shoot-, on Nicotiana glutinosa leaves, cause unknown, 2
Endoconidiophora spp., in chemically treated lumber stain, 272
Entomophthora spp., insects parasitized by, 10
Entomosporium maculatum, overwintering on dead leaves, 18
Epidemiology, of plant diseases, 215
Errata, Phytopathology (vol. 30), 377
Erysiphe spp., conidia, substances stimulatory to, 865
  cichoracearum, on cantaloupe, resistance of host strains to races of, 863
    on lettuce, resistance inheritance, 534
    physiologic races, 539
    potato powdery mildew due to (?), 357
    thiamin effects on, 863
  solani, on potato, 358
Esters, losses in sterilization, 153
Etch, tobacco-, virus, quantitative measurements, 12
Ethylene chloride, nematode control by soil treatments with, 818
Evans, A. W., (463), 469
Experimental technique (see under Technique)
Eye spot, lemon grass Helminthosporium, 186
  Napier grass Helminthosporium, 855

Factorial designs, disease data in complex-, handling and interpreting, 26
Fagopterum esculentum, virus inoculations, 579
Fagus spp., Phytophthora bleeding canker, 863
    control by tree injection, 12
  grandifolia, Stereum murrayi on, 82
Fawcett, Howard S., 356, 861
Felix, E. L., 9
Fertilizers, effect on apothecial development in Sclerotinia, 1023
  wheat yields as influenced by seed disinfection and, 385
Ficivir caricae n. gen. and sp., for fig mosaic, 563
Fig, Cephalosporium leaf spot, 771
  mosaic, distribution and notes, 561
    Ficivir caricae binomial proposed for, 563
Fischer, George W., 674
Fisher, Herbert, (10)
Flax, Fusarium wilt, resistance to, 4
    variations in fungus, 4
  rust, physiologic races, pathogenicity of aeciospores from selfing and crossing, 852
Flies, fungi parasitizing, 10
Flint, L. H., 569
Flor, H. H., 852
"Flordo," tobacco downy mildew controlled by, 286
Fluorescence, of diseased potatoes, nonspecificity, 569
  potato bacterial ring rot differentiated by, 10
Fogelberg, S. O., (14)
Fomes fomentarius, morphology and biology, 11
    wood decay by, 11
Foot rot, annual stock Rhizoctonia, 87
  wheat Helminthosporium, 383
Forage crops, diseases of northern U.S.A., book review, 200
Forbes, I. L., 767
Forest pathology, demonstration specimens, storage and mounting technique, 763
Forestiera neomexicana, bacterial olive knot on (exp.), 362
Fragaria virus-5, strawberry stunt (new) due to, 851
Frampton, Vernon L., 9, 1040
Freitag, J. H., 8
Friend, S., 861
Frog eye, tobacco Cercospora, 97
Fruit spot, guava algal-, 95
Fruits (see also Trees, orchard fruit-; Stone fruits)
  from Hawaii, quarantines on, 570
Fulton, Robert W., 575
Fumigants, soil-, for rodent control, 23
Fungus(i), air-borne populations, appraisal technique, 201
  antagonisms among, 991
  on barnyard grass, weevil relations, 832

- growth rates, photoelectric determination method, 448
- inheritance, in Sphacelotheca, 254
  - in Tilletia, 80
  - in Venturia, 1142
- inoculum preparation by mechanical liquefier, 566
- insects parasitized by, 10
- mealy bug parasitized by, 5
- mycorhizal, of conifers in Germany, 567
- pine root-, crown and butt infections in artificial vs. natural stands, 25
- in pine trunks in natural vs. artificial stands, 25
- plant disease-, book review, 100
- root-infecting forms, solution-culture inoculation methods, 549
- thermal death point, genetic role, 264
  - growing conditions vs., 264
- toxicities of compounds to, technique of measurement, 24
- toxicity ratings of compounds by different, 16
- toxins of, 991
- wood-inhabiting, growth rates compared, 825
- wood-rotting, differentiation methods, 6
- wood-staining, in chemically treated lumber, 270

Fungicidal action, of gliotoxin, 1001

Fungicides, assay tests, hanging-drop method of applying, 1109
  - spore-germination method, 1108
- copper-, Bordeaux effect on water loss from inert surfaces, 865
  - compatibility of "inert" ingredients of cuprous oxide, 8
  - deposition as influenced by Al, 860
  - downy mildew conidial stimulation by dilute, 865
  - effectiveness as influenced by Al, 860
  - -soap spray, for tobacco downy mildew, 286
  - solubility as influenced by supplements, determination method, 17
  - tomato tests with, 10
- effect on sour cherry yields and quality, 16
- eradicant, for apple scab, 13, 296
- Helione orange, Phytophthora canker of hardwoods controlled by injection of, 12
- iodine, in potato scab and ring rot control, 954
- mercurial-, cabbage clubroot control by, 18
  - cotton seed treatment by organic-, mode of action, 768
  - for potato tubers, acidulated $HgCl_2$, 812
  - wheat (disease-free and infected) seed as influenced by dust-, 379
- organic, sterilization for use in culture solutions, methods quantitatively compared, 149
- protective value, laboratory vs. field prediction, 12
- for rose powdery mildew, as influenced by wetting agents, 16
- silver sprays, fungicidal and adhesive properties, 17
- "Spergon," as seed protectant for canning peas, 20
- spore-germination tests of, duration of inhibition in, 24
- sprays, coverage index vs. inoculum potential, measurement technique and relations, 7
- sulphur, for downy mildew, evaluation, 865
  - effect on carotene in white-clover leaves, 556
- toxicity ratings by different fungi, 16
- for wound dressings, 24

Fusarium spp., on barnyard grass, weevil relations, 836
  - on cotton, seedling and boll injury association, 160, 769
  - in damping-off of longleaf pine, 1012
  - lupine hypocotyl rot by Martiella group-, distribution and general study, 771
  - solution culture infection methods for wilt-inducing, 549
  - sweet potato wilt due to, cross inoculations, 21
  - tobacco wilt due to, cross inoculations, 21
  - wilt forms and hosts, 1
- bulbigenum var. lycopersici, pathogenic and culture variations in single-spore isolates of strains, 103
  - on tomato, epinasty as early symptom, 281
- culmorum, on wheat, seed treatments for, by dust mercurials, 379
- eumartii, on potato, diagnosis, 569
- lini, culture and parasitic variations in, 4
- moniliforme, on cotton, seedling survival statistics, 1088
- nivale, turf snowmold caused by Typhula itoana vs., 940
- oxysporum f. **barbati** n. f., sweet william wilt caused by, 1056
  - var. cubense, growth in soil, 91
    - as influenced by sodium nitrate, 564
- perniciosum, cultural characters and variations, 600
  - life history and dissemination, 611
  - mimosa tree wilt caused by, distribution and general study, 599
- vasinfectum, on cotton, pathogenicity of isolates to vars. compared, 770
  - regional varietal studies, 770
    - nematode distribution in, 771
    - and phosphorus relations, 771
  - varietal reactions to root knot and, 1099

Fusicladium saliciperdum, willow blight association rare in Canada, 1056

Fusicoccum amygdali, peach canker caused by, new to America, 10

GAINES, J. G., (6)

Gardenia, bacterial leaf spot (new), 192
- fungus (new) leaf spot (Tuberculariaceae), 2

gardeniae (Phytomonas), 194
Garlic, smut resistance, 9
Gas, illuminating-, London plane tree rosy canker associated, 349
GENAUX, CHARLES M., 99
Genetics (see also Breeding; Inheritance) factors in strawberry yellowing defect due to, 1
Geranium, invading overgrowths in, 15
Gibberella saubinetii, barley infected by, emetic principle in, concentration and characterization, 871
  on corn, spread in pith, 20
Giddings, N. J., 8, 861
Gilbert, William Williams, biographical sketch, portrait and bibliography, 961
  necrology, 375
GLICK, P. A., 201, 216
Gliocladium fimbriatum, toxin, extraction technique, 993
    production, stability, and activity, factors influencing, 991
Gliotoxin, extraction technique, 993
  factors in production, stability and activity, 991
  fungicidal properties, 1001
Glomerella cingulata, camellia anthracnose caused by (?), 6
  gossypii, on cotton, boll rot association of Bacterium malvacearum and, 24
    seedling and boll infection, 158, 769
    seedling survival statistics, 1088
Gloxinia, boron deficiency disease, cause, description and control, 467
Glucose, in damping-off control, 857
Glucosides, losses in sterilization, 153
GOLDSMITH, G. W., 452
Golf courses, turf disease (new), Curvularia or Helminthosporium causing, 31
Goodwin, M. K., 8
Gossypium hirsutum, Verticillium alboatrum infection (exp.), 1134
GOTTLIEB, MANFRED, 944
GRANOVSKY, A. A., 9
Grape, muscadine-, leaf spots and berry rots, 767
  Pierce's vine disease, development and spread, 862
Graphium rigidum, in chemically treated-lumber stain, 272
Grass(es), barnyard- (see Echinochloa crusgalli)
  "bends" disease (new), cause unknown, 674
  diseases of forage-, control by selection and hybridization, 1
  eastern snowmold, Typhula itoana causing, 940
  fungus turf disease (new), Curvularia or Helminthosporium causing, 24
  Helminthosporium eye spot, reactions of Napier and Merker grasses and crosses to, 855
  killing by basidiomycete, 1058
  smuts, sori from ovarial and staminal tissues, variations in, 1046
    variations in cultures of, 761
GREANEY, F. J., (379)
GREATHOUSE, GLENN A., 149
GROVES, A. B., (1047)
Growth rates, determination in fungi, photoelectric method, 448
Growth substances, in aphid extracts, 15
  in cabbage leaf extracts, 15
  crown gall relations, 19
  effect on carnation cuttings, 14
  invading overgrowths induced by bacteria vs., 15
Guava, algal leaf and fruit spot, 95
Guignardia **n. sp.**, grape (muscadine) berry rot caused by, 768
  bidwellii, muscadine grape black rot fungus differing from, 767
Gum pockets, in citrus, 861
Gymnoconia spp., flexuous hyphae in, 4
Gymnosporangium clavipes, flexuous hyphal fusions in, 4

HAENSELER, C. M., 10
Hairy root, comparative physiology of bacteria of, 964
HANSING, E. D., 1043
HANSON, E. W., 832
Hardwoods, defects in oak-hickory stands in Iowa, 99
  heartrots, differentiation methods, 6
  lumber, staining in chemically treated, 272
  Phytophthora canker, control by tree injection, 12
  Stereum murrayi associated heartrot and cankers of, 82
Harposporium **diceraeum** n. sp., nematodes parasitized by, 799
  helicoides n. sp., nematodes parasitized by, 794
  oxycoracum n. sp. nematodes parasitized by, 796
HARRAR, J. G., (5), 10
HARRISON, A. L., 749
HARTER, L. L., 26, 961
HARVEY, R. B., 10
HAWKINS, B. S., (1)
Heartrot(s), of black cherry, differentiation, 6
  by Fomes fomentarius, 11
  hardwood-Stereum murrayi association, 82
  oak-, differentiation, 6
Heat therapy, of aster yellows, 14
HEBERT, T. T., (770)
HEDGES, FLORENCE, 10
Helianthus annuus, tobacco ring-spot inoculations, 580
Helichrysum bracteatum, Verticillium wilt, 1130
Helione orange, Phytophthora bleeding canker of hardwoods controlled by injection of, 12
Helminthosporium spp., on barnyard grass, weevil relations, 836
  grass disease caused by (?), 24
  geniculatum, in chemically treated lumber stain, 272
  maydis, corn disease caused by, distribu-

    tion, morphology and general study, 508
  physiologic races, 508
  ocellum, lemon grass eye spot caused by, 186
  sacchari, reactions of Napier and Merker grasses and their crosses to, 855
  sativum, on wheat, seed treatments by dust mercurials for, 383
Henderson, R. G., 10
Hepting, George H., 99
Heterodera marioni, biology and giant cell development, 642
  on cotton, distribution in regional wilt plats, 771
    numerical evaluation of infestation, 1099
    resistance to, 771
    varietal resistance to Fusarium wilt and, 1099
  effect on tomato wilt, 749
  feeding before and during root entry, 862
  parasitism in leaves and stems, 634
  soil moisture relations, 862
  soil treatments for, 821
  in tobacco plant-beds, soil treatments for, 6
  wound infestation, 641
Heuberger, J. W., 11, 12
Hewitt, Wm. B., 862
Hickory-oak stands, defects in, in Iowa, 99
Hilborn, M. T., 11, (22)
Hildebrand, E. M., 11, 353, 355
Hirsutella spp., insects parasitized by, 10
Holly, Rhizoctonia defoliation of cuttings, and control, 6
Holmes, Francis O., 12, 1089
Holton, C. S., 74
Hopperstead, S. L., 12, (13)
Hops, downy mildew, sporulation injury by, 841
Horne, W. T., (561)
Howard, Frank L., 12
Hoyman, William G., 871
Huber, Glenn A., 712, (1023)
Hutchins, Lee M., (19), (860)
Hybridization, of Tilletia spp., 80
Hydrogen-ion concentration, effect on X-ray inactivation of virus, 862
Hyphomycetes, nematodes (free-living terricolous) parasitized by, 773

Iberis umbellata, Rhizoctonia foot rot (exp.), 89
Illuminating gas, London plane tree rosy canker associated, 349
Immunity (see also Resistance)
  acquired, virus use of term, 1061
    viruses in relation to, 1059
  against viruses, competition vs., 23
Inclusion bodies, of plant viruses, 760
  tobacco mosaic-, leaf-starvation effects on, 1121
Infections, leaf-, by bacteria, watersoaking relation, 6
  mechanism, micrugical study method, 11
  nutrient effects on, 10
Inheritance (see also Breeding; Genetics)
  of bean leaf variegation, 26
  fungus-, in Sphacelotheca, 254
    in Tilletia, 80
    in Venturia, 1142
  lettuce, resistance to Erysiphe powdery mildew, 534
  wheat, stem rust resistance, 558
Inoculation method, mechanical liquefier for inoculum preparation, 566
Inoculum potential, spray coverage index vs., measurement and relations, 7
Insecticides, arsenical-, compatibility of ''inert'' ingredients of calcium arsenate, 8
Insectivirus n. gen. (viruses), 23
Insects, air-borne populations, appraisal techniques, 201
  economic, fungi parasitizing, 10
Insects as vectors, aster yellows by leaf hopper, mechanical transmission and multiplication in vector, 120
  book review, 98
  cereal root rot and basal stem rot by weevils, 837
  cowpea mosaic by aphids, 423
  of cucurbit viruses, 8
  Dutch elm disease, by bark beetles, 19
    by European elm bark beetle, 657
  Fusarium perniciosum by ambrosia beetles, 611
  grass root rot and basal stem rot by weevils, 832
  potato pit-scab role, 9
  potato yellow dwarf by clover leaf hopper, hereditary variation in, 3
  strawberry stunt by aphids, 850
  sugar beet curly top, leaf hopper relations to virus strains, 8
Iodine, crown gall on almonds controlled by, 956
  in potato ring rot and scab control, 954
Irish, Charles F., (22)
Iron deficiency, in cotton, symptoms, 771
Iron salts, chlorosis of pin oak controlled by, 18
Ironwood (see Ostrya virginiana)

Jenkins, Anna E., 13, 194
Jenkins, Wilbert A., 767, 948
Johnson, E. M., (6)
Johnson, Folke, 13, 649
Johnson, James, 679
Johnson, John W., 1035
Johnson, H. W., (1)
Johnson grass (See Sorghum halepense)
Johnston, J. C., (860)
Jolivette, J. P., (23)
Jones, Elon K., 197
Jones, F. R., 765
Jones, Winston W., (340), 570
Juglans spp., Chalaropsis stem infection (exp.), 859

Kadow, Kenneth J., (8), 13, 23
Kalanchoë, invading overgrowths in, 15
Keitt, G. W., 13, 14, 296, 1142
Kendrick, James B., 14
Kentucky blue grass (see Poa pratensis)

Kernkamp, M. F., 333, (761), 1051
Kincaid, Randall R., 286
Kirby, R. S., 14, (24)
Klotz, L. J., 860, (864)
Köck, Gustav, biographical sketch, bibliography, and portrait, 867
Kreutzer, W. A., 907
Kramer, M., (430)
Kuenzel, John G., (99)
Kunkel, L. O., 14

Lackey, C. F., 861
Lactarius spp., mycorhizal, on conifers, 568
Lactuca spp., Erysiphe powdery mildew, 534
    Marssonia panattoniana infecting, 17
Lambert, E. B., Chairman et al., 201
Langford, M. H., (296), (1142)
Large, John R., 768
Larix europea, mycorhizas of, in Germany, 568
Larson, R. H., 14
Laskaris, Thomas, 254
Leach, J. G., (7), 98
LeClerg, E. L., 49, 274
Leaf hopper(s), aster-, as vector of aster yellows, mechanical transmission and multiplication in, 120
  clover-, as vector of potato yellow dwarf, hereditary variation in, 3
  fungi parasitizing, 10
  as vectors of sugar beet curly top, relations to virus strains, 8
Leaf roll, potato-, yield losses by, 3
Leaf spot(s), of Bromus inermis, Selenophoma-induced, 953
  cherry Coccomyces, 22, 1047
  cotton angular-, 4
    boll rot association of organisms of anthracnose and, 24
  fig Cephalosporium, 771
  gardenia bacterial (new), 192
  gardenia fungus-, 2
  of grape (muscadine), 767
  guava algal-, 95
  pea-, Cercospora lathyrina-induced, 1031
  peanut-, 16, 770
  pear Entomosporium, 18
  poplar-, Mycosphaerella populicola causing, 250
    Mycosphaerella populorum causing, 242
  rice Cercospora, 19
  strawberry Mycosphaerella, 239
  strawberry Mycosphaerella purple, 225
Leaves, nematodes parasitizing roots vs., morphological comparisons, 645
  root knot parasitism in, 634
  transpiring, vapor pressure gradient above, 1040
  water-soaking, effect on bacterial invasion, 6
Lecanosticta acicola, imperfect stage of Systremma acicola, 73
  on pine, 73
Leek, smut resistance, 9
Lefebvre, C. L., (1), (1043)
Legumes, basidiomycete killing, 1058
  Rhizoctonia root rot, 674
Lehman, S. G., 768
Lemon, red scale on, Bacillus "C" destroying, 864
Lemon grass (see Cymbopogon citratus)
Leptosphaeria salvinii, sclerotia, longevity, 279
Lethal dosage, of compounds, fungus ratings of, 16
Lettuce, Erysiphe mildew, resistance inheritance, 534
  Marssonia shot-hole, hosts and overwintering, 17
Levine, Michael, 15
Lewis, F. H., (16)
Li, C. C., (753)
Lightle, Paul C., 15
Lightning injury, to tomato, symptomatology, 1017
Lily-of-the-valley, Ascochyta majalis infecting, and distribution, 13
Lime, effect on apothecial development in Sclerotinia, 1023
Line-pattern virosis (new), of stone fruits, distribution, relationships and general study, 1004
Linford, M. B., 634, 862
Ling, Lee, 264, 617, 664
Link, G. K. K., 15
Locke, S. B., (964)
Loegering, William Q., 952
Longrée, Karla, (1040)
Lorenz, Rolland C., (82)
Lumber (see also Wood)
  stain in chemically treated, fungi associated, 270
Lupines (see Lupinus)
Lupinus spp., Fusarium hypocotyl rot, distribution and general study, 771
Lutman, B. F., 702
Lyneis, Mary M., (964)
Lysis, heritable, in germinating Sphacelotheca chlamydospores, 254

McCallan, S. E. A., 16, (24)
McCallum, A. W., (1056)
McClellan, W. D., 16
McKelvey, J. J., (10)
McKelvey, J. J., Jr., (5)
McKinney, H. H., 1059, (1140)
McLean, D. M., 420
McWhorter, Frank P., 760
Machacek, J. E., 379
Macrochelus sp., potato pit-scab role, 9
Macrophoma **n. sp.**, grape (muscadine) berry rot caused by, 768
Macrosiphum pisi, as vector of cowpea mosaic, 423
  solanifolii, as vector of cowpea mosaic, 423
Macrosteles divisus, as vector of aster yellows, mechanical transmission and multiplication in, 120
maculicola (Phytomonas), 347
Magnesium, deficiency in cotton, symptoms, 771

  deficiency and excess, in citrus, 861
malvacearum (Bacterium), 24
  (Phytomonas), 4
Manganese deficiency, in citrus, 861
  in cotton, symptoms, 771
Manns, T. F., (12)
Maple (see Acer)
Marmor spp., activity measurement, local lesion method, accuracy, 21
    transmission via dodder, 649
  cerasae n. sp., mottle virosis of Prunus spp., 467
  cucumeris var. judicis, relation to commelina mosaic virus, 756
  erodens, quantitative measurement of, 12
  lineopictum n. sp., line-pattern virosis of stone fruits, distribution, relationships and general study, 1009
  medicaginis, concentration in tobacco at intervals after inoculation, 410
    protein of, purification and properties, 394
  nerviclarens n. sp., vein clearing viroses of Prunus spp., 467
  tabaci, inactivation, nitrogen relations, 21
    virus protein synthesis vs. leaf chromoprotein and cell metabolism, 978
    var. **plantaginis** n. var., from Plantago spp., 1097
  vastans, longevity and purification, 3
Marsh, Paul M., (9)
Marshak, Alfred, 862
Marssonia panattoniana, lettuce shot-hole caused by, hosts and overwintering, 17
Martin, E. A., (7)
Martin, W. J., 761, (1051)
Matthiola incana, Rhizoctonia foot rot, 87
Maxwell, L. R., (175)
May, Curtis, 15, 349
Mealy bugs, fungi parasitizing, 5, 10
Meinecke, E. P., 289
Melampsora spp., flexuous hyphae in, 4
  lini, hybrid, heteroecism(?) in, 854
    physiologic races, pathogenicity of aeciospores from selfing and crossing, 852
Melampsorella spp., flexuous hyphae in, 4
Melanconium **n. sp.**, grape (muscadine) berry rot caused by, 768
Melanophila californica, larval galleries, effect on liquid conduction in wood, determination, 1035
Melilotus spp., basidiomycete killing, 1058
    resistance to fungus parasites, 766
    Rhizoctonia root rot, distribution and importance, 674
Mercurials, fungicidal- (see under Fungicides)
Meredith, Clifford H., 91, 564
Meria **coniospora** n. sp., nematodes parasitized by, 792
Merulius lacrymans, growth rate, comparisons, 826
Metabolism, plant cell-, relation to synthesis of virus protein, 978
Metcalf, Haven, biographical sketch, portrait and bibliography, 289
  necrology, 376
Methyl bromide, Colletotrichum infections (latent) diagnosed by, 570
Metropolitan bent grass (see Agrostis palustris)
Mice, orchard injuries by, control, 23
Microorganisms, air-borne populations, appraisal techniques, 201
  virus inactivation by growth products of, 690
Microsphaera spp., conidia, substances stimulating, 865
Micrugical method, cellular applications, 11
Middleton, G. K., 351
Middleton, John T., 863
Mildew(s), cantaloupe downy-, 769
  cantaloupe powdery-, 863
  downy-, control by sulphur fungicides, evaluation, 865
    sporulation injury associated, 741
  lettuce Erysiphe, 534
  oats downy-, 768
  potato Erysiphe, 357
  powdery-, conidia, substances stimulatory to, 865
  rose powdery-, 16
  tobacco Peronospora, 286
Miles, L. E., 768
Miller, H. J., (1047)
Miller, Lawrence I., 16
Miller, P. A., 863
Miller, Paul R., (24), (158), 769
Millet, Phoma terrestris infecting (exp.), 913
Milliron, H. E., (832)
Mills, W. D., 16, (355)
Mimosa tree (see Albizzia julibrissin)
Mites, potato pit-scab hole, 9
Molds, toxins of, 991
Moles, plant injuries by, control, 23
''Monosporous,'' ''single-spored'' vs., 471
Mook, Paul V., (23), (349)
Moore, Elizabeth Jane, (448), (452)
Moore, J. D., (13)
Morris, H. E., (1)
Mosaic(s), acquired immunity relations, 1059
  alfalfa-, symptoms, as influenced by virus concentration, 418
    virus, activity measurement, local lesion method, accuracy, 21
      concentration in tobacco at intervals after inoculation, 410
      extraction technique, 411
      inactivation *in vivo*, 415, 419
      purification and properties of protein of, 394
  bean-, virus, Bacterium phaseoli associated, and nutrient relations, 10
      behavior in roots vs. aerial parts of exp. hosts, 577
  bean-leaf variegation resembling, 26
  commelina-, virus, hosts and classification, 756
  cowpea, distribution, hosts, insect transmission and general study, 420
    virus properties, 424

  cucumber-, virus, relation to commelina mosaic virus, 756
    virus-1, behavior in roots vs. aerial parts of exp. hosts, 577
  cucurbit-, transmission via dodder, 13, 652
  elm-, new mottled-leaf-, 22
  fig-, distribution and notes, 561
    Ficivir caricae binomial proposed for, 563
  peach-, strain incidence and eradication in Colo., 4
    varietal reactions to, 329
    virus, hosts, in W. Colo., 322
  peach rosette-, case report, 353
  potato rugose-, virus, movement and route, 7
  prune-, peach infection (exp.), 355
  squash-, virus, insects transmitting, and properties, 8
  tobacco-, changes in N and virus content of detached leaves in darkness, 1117
    inclusion bodies, leaf-starvation effects on, 1121
    resistance to, Ambalema vs. glutinosa types, significance, 1140
    transmission via dodder, 13
    virus, activity measurement, local lesion method, accuracy, 21
      Brazilian bean vars. as indicators, 430
      effect on cellular oxidation, 25
      inactivation, nitrogen relations, 21
        by X-rays vs. pH, 862
      inactivator from yeast, 864
      multiplication, host growth vs., 861
        P-nutrition vs., 861
      new var. from rib grass, 1089
      -protein, synthesis vs. leaf chromoprotein and cell metabolism, 978
      starvation effects on stream double refraction, 1121
      transmission via dodder, 653
    virus-1, behavior in roots vs. aerial parts of exp. hosts, 577
  white clover-, virus, transmission via dodder, 13
    virus-1, transmission via dodder, 654
Mottle, peach-, virus-induced, 860
MOULTON, JAMES E., (15)
MURPHY, D. F., (8)
Mycology, plant disease-, book review, 100
Mycorhizas, on conifers, in Germany, 567
    nutritional and ecological relations, 567
Mycosphaerella **n. sp.**, as Cercospora brachypus perfect stage, 767
  fragariae, Ramularia tulasnei as imperfect stage, 239
  louisianae n. sp., strawberry purple leaf spot due to, distribution and general study, 236
  populicola n. sp., poplar leaf spot due to, distribution, hosts and general study, 250
    Septoria populicola as imperfect stage, 241
  populorum n. sp., poplar leaf spot due to, distribution, hosts and general study, 242
    Septoria musiva as imperfect stage, 241
Myriogenospora sp., sugar cane infection and distribution, 564
Myzus persicae, extracts of cabbage leaves and, effect on oats coleoptile test, 15
    as vector of cowpea mosaic, 423

Nanus **cupuliformans** n. sp. (=strawberry stunt virus), 851
Narcissus nematode, soil treatments for, 818
NEAL, D. C., 769
Necrosis, giant cactus bacterial (new), 15
  of snap-bean tissues, 767
  tobacco-, virus activity measurement, local lesion method, accuracy, 21
Needle disease, pine Systremma brown spot-, 61
Nemacides, volatile, soil treatment by, 818
Nematoctonus n. gen. (Hyphomycetes, Mucedinaceae), 779
  leiosporus n. sp., nematodes parasitized by, 779
  tylosporus n. sp., nematodes parasitized by, 779
Nematodes (see also Acrobeloides; Anguillulina; Heterodera; Plectus; Pratylenchus; Rhabditis; Rotylenchus)
  on cotton, distribution in regional wilt plats, 771
  feeding before and during root entry, 862
  free-living terricolous, Hyphomycetes parasitizing, 773
  soil treatment with volatile liquids for, 818
  species parasitizing roots vs. aerial parts, morphological comparisons, 645
NEWHALL, A. G., 17
Nicandra physaloides, virus inoculations, 579, 580
Nicotiana spp., commelina mosaic virus infecting (exp.), 756
    crown gall on, temp. relations, 976
    rib grass mosaic infecting, 1089
    virus inoculations, 579, 580
  glutinosa, shoot enations on leaves, cause unknown, 2
NIELSEN, L. W., 17
NIKITIN, A. A., 17
Nitrates, effect on damping off, and control by glucose, 858
Nitrogen nutrition, effect on tobacco mosaic virus, 21
Nitrogen starvation, in citrus, 860
  effect on plant-cell respiration, 981
Nomenclature, viruses, system suggested for citrus-, 356
NORRIS, D. O., (181)
Nurseries, rodent damage to, control, 23
NUSBAUM, C. J., 769, 770
Nutrition, nitrogen-, effect on tobacco-mosaic virus, 21
Nymphaea tuberosa, Pythium marsipium from leaves of, 506

Oak, California live- (see Quercus agrifolia)
  live- (see Q. virginiana)
  pin- (see Q. palustris)
Oak-hickory stands, defects in, in Iowa, 99
Oats-, coleoptile test, as influenced by extracts of cabbage leaves and aphids thereon, 15
  covered smut, susceptibility of seed lots differing, 672
  downy mildew, in Miss., 768
  loose smut, susceptibility of seed lots differing, 673
  Phoma terrestris infecting (exp.), 913
  susceptibility of seed lots to smuts differing, basis, 672
Okra, Fusarium wilt, and cross inoculations, 1
  virus inoculations, 579, 580
Olea spp., bacterial olive knot on (exp.), 362
Olive knot, bacterial, exp. hosts, 361
Olson, Alver J., 1063
Onion, downy mildew, sporulation injury by, 741
  Phoma pink root, host-parasite relations, 907
  smut resistance, 9
Orange, Chalaropsis stem infection (exp.), 859
Orchard fruits (see Trees, orchard fruit-)
Organic compounds, sterilization for use in culture solutions, methods quantitatively compared, 149
Osmanthus spp., bacterial olive knot on (exp.), 362
Ostrya virginiana, Stereum murrayi on, 82
Overgrowths (see also Crown gall; Tumors)
  invading plant-, bacterial vs. growth-substance-induced, 15
Owens, C. E., (469)
Oxidation, cellular, tobacco mosaic virus effect on, 25

Palm, date- (see Phoenix dactylifera)
Palmer, Ralph G., 18
Panama disease, banana Fusarium, 91, 564
Papaya, Colletotrichum latent infection, diagnosis by methyl bromide, 570
Parker, K. G., (19), 657
Parris, G. K., 340, 570, 855
Paspalum dilatatum, ergot on, breeding for resistance, 1
Pathogens, inoculum preparation by mechanical liquefier, 566
Pathogens, root-infecting forms, solution-culture inoculation methods, 549
Pathology, forest-, demonstration specimens, storage and mounting technique, 763
Pea, canning-, root rots, control by "Spergon" seed protectant, 20
  Cercospora lathyrina leaf spot, 1031
  Phoma terrestris infecting (exp.), 913
  Rhizoctonia from potato vs. sugar beet on, 277
  Rhizoctonia root rot, 674
  root knot parasitism in leaves and stems, 636
Peach, bacterial shot hole, overwintering in buds, 12
  buckskin, reaction of cherry vs., 864
    symptoms, 917
  Chalaropsis stem infection (exp.), 859
  Fusicoccum canker, new to America, 10
  mosaic, strain incidence and eradication in Colo., 4
    varietal reactions to, 329
    virus, hosts in W. Colo., 322
  mottle, virus-induced, 860
  ring-rot virosis, 860
  rosette-mosaic, case report, 353
  Sclerotinia spp. on, 724
  virosis (new) resembling X-disease, 19
  wart, virus-induced, 859
  X-disease-like virosis, 860
Peanut, leaf spot(s), control, by sulphur dust, 16
    by copper-sulphur dusts and copper sprays, 770
  mineral deficiency (?) disease, 948
Pear, Chalaropsis stem infection (exp.), 859
  Chinese sand-, Entomosporium maculatum overwintering on dead leaves, 18
  Clitocybe root rot (exp.), 93
Pecan, Elsinoë nursery blight, fungicidal control, 768
Pelargonium hortorum, Armillaria root rot, temp. relations, 3
Penicillium spp., on barnyard grass, weevil relations, 836
Pentstemon spectabilis, Ascochytella stem spot (new), 194
Pepper, bacterial spot, variations in, 753
  Phoma terrestris isolated from, 914
Peppery spot, cauliflower bacterial-, 347
Peronoplasmopara cubensis, copper fungicidal action as influenced by Al, 860
Peronospora spp., sulphur fungicide effects on, 865
  destructor, on onion, sporulation injury by, 741
  effusa, on spinach, sporulation injury by, 741
  tabacina, on tobacco, control by copper-soap spray, 286
  trifoliorum, alfalfa resistance to, 766
Perotti, Renata, 100
Peterson, A. G., (9)
Peterson, P. D., 1108
Petty, M. A., (333)
Petunia violacea, virus inoculations, 579, 580
phaseoli (Bacterium), 10
Phaseolus virus-2, isomeric crystals produced by, 760
Phenols, losses in sterilization, 153
Phillyrea decora, Phytomonas savastanoi inoculations on, 362
Philodendron giganteum, cuttings, abnormal rooting vs. Ca-deficiency, 844
Phoenix dactylifera, rhizosis of, Ceratostomella association and general study, 1123
Pholiota adiposa, growth rate, comparisons, 826
Phoma terrestris, onion pink root due to, host-parasite relations and hosts, 907

Phomopsis sp., tomato fruit rot due to, 24
  tumors of elm and maple due to, 544
Phosphorus deficiency, in cotton, symptoms, 771
Photoelectric method, growth rate determination in fungi by, 448
Phragmidium spp., flexuous hyphae in, 4
Phylactinia spp., conidia, substances stimulating, 865
Phyllody, anatomy and morphology in bean and tomato vars., 168
Phymatotrichum omnivorum, ammonium-salt toxicity to, mechanism, 926
  on cotton, inoculations in sand culture, technique, 358
    resistance to, field selection tests for, 452
  pH relations, 930
Physalospora miyabeana, willow blight association in Canada, 1056
Physiologic disease, cherry "physiological yellow leaf" not, 355
  potato spindling tuber as, 346
Physiologic races of: bean rust, 26
  Erysiphe cichoracearum, 539, 863
  Fusarium lini, 4
  Helminthosporium maydis, 508
  Melampsora lini, 852
  Phytomonas vesicatoria, 753
  Puccinia triticina, 5
"Physiological yellow leaf," cherry (sour), distribution, history and virus etiology, 355
Phytomonas (see also Bacterium)
  barbareae n. sp., Barbarea vulgaris black rot due to, 348
  campestris, in crucifers, notes, 347
  gardeniae n. sp., gardenia leaf spot due to, 194
  maculicola, in cauliflower, notes, 347
  malvacearum, wind dissemination, 4
  pruni, in peach, overwintering in buds, 12
  rhizogenes, comparative physiology, 964
  savastanoi, oleaceous hosts (exp.), 361
  sepedonica, in potato, control, by iodine seed treatment, 954
      "seed" treatments for, 7
    differentiation by fluorescence, 10, 569
    dissemination, 14
    tomato infection (exp.) by, 14
  stewartii, in corn, susceptibility and age relations of dent inbreds, 7
  tumefaciens, in almond trees, chemical eradication, 956
    comparative physiology, 964
    crown gall growth substance relations, 19
    host-parasite and temp. relations on various hosts, 972
    pathogenicity, basis, 964
    in sunflower, secondary tumor and strand development in, 135
    in tomato, infection mechanism, micrugical study, 11
    tumor formation by, mechanism of primary vs. secondary, 148
  vesicatoria, variations in strains, 753
PHYTOPATHOLOGY, errata (vol. 30), 377
Phytopathology, manual, review, 960
Phytophthora cactorum, bleeding canker of hardwoods due to, control by tree injection, 12
  infestans on potato, control by silver sprays, 17
Phytoviraceae new family (viruses), 23
Phytovirus n. gen. (viruses), 23
  nicomosaicum var. **vulgare** n. nom. (=tobacco mosaic virus), 23
Picea abies, mycorhizas of, in Germany, 568
Pierce's grapevine disease, development and spread, 862
Pine, longleaf- (see Pinus palustris)
  -lumber, staining in chemically treated, fungi associated, 272
  western white- (see Pinus monticola)
  western yellow- (see Pinus ponderosa)
Pineapple, root knot parasitism in leaves and stems, 637
Pink root, onion Phoma, 907
Pinus spp., Cunninghamella root disease (new), 1063
    fungi in trunks in natural and artificial stands, 25
    mycorhizas of, in Germany, 568
    root, crown and butt fungi, susceptibility in artificial vs. natural stands, 25
    Systremma brown spot needle disease, distribution and general study, 61
  monticola, Armillaria mellea from, variations in culture, 803
  palustris, damping-off symptoms, associated fungi and control, 1011
  ponderosa, liquid conduction route in, silver nitrate stain showing, 1035
PIRONE, P. P., (6), 18, 100, 192, 960
Pisum virus-2, isomeric crystals produced by, 760
Pit-scab, potato Actinomyces, 9
PLAKIDAS, A. G., 18, 93, 225
Planetree (see Platanus)
Plantago lanceolata, mosaic (new var.) from, 1089
  major, mosaic (new var.) from, 1089
Plantain, broad-leaved (see Plantago major)
Plants, virus behavior in roots vs. aerial parts, 575
Plasmodiophora brassicae, on cabbage, control by mercurials, 18
Platanus spp., Ceratostomella canker, transmission and control, 23
    London plane tree var., rosy canker, illuminating-gas injury associated, 349
Plectus spp., Harposporium oxycoracum parasitizing, 797
    Nematoctonus leiosporus parasitizing, 780
  parvus, Acrostalagmus bactrosporus parasitizing, 782
    Acrostalagmus obovatus parasitizing, 784
    Cephalosporium balanoides parasitizing, 786

    Harposporium diceraeum parasitizing, 799
    Spicaria coccospora parasitizing, 787
Pleurotus ostreatus, growth rate, comparisons, 826
  ulmarius, growth rate, comparisons, 826
Plum, Abundance, virus diseases of, 1004
  myrobalan, peach ring rot grafted into, 860
  peach mosaic on, 323
Plummer, Bernie E., Jr., 812
Poa annua, fungus disease (new), Curvularia or Helminthosporium causing, 24
  pratensis, Helminthosporium leaf spot, breeding for resistance, 1
Podosphaera spp., conidia, substances stimulating, 865
Poinsettia, Sphaceloma scab, pathology and etiology, 947
Pollen, air-borne populations, appraisal techniques, 201
Poplar (see Populus)
Populus spp., Mycosphaerella populicola leaf spot (new), 250
    Mycosphaerella populorum leaf spot (new), 242
Portraits, Collins, James Franklin, 475
  Gilbert, William Williams, 961
  Köck, Gustav, 867
  Metcalf, Haven, facing p. 289
Portulaca oleracea, root knot parasitism in leaves and stems, 636
Potassium deficiency, in cotton, symptoms, 771
Potato, Actinomyces pit-scab, insect and saprophyte role, 9
    control by iodine, 954
    cyto- and histopathology, 702
  bacterial ring rot, control, by iodine "seed" treatment, 954
      "seed" treatments for, 7
    diagnosis, by fluorescence, 10
      nonspecificity, 569
    dissemination, 14
    tomato exp. infection, 14
  blue-stem, varietal reactions to, 5
  Erysiphe powdery mildew of, 357
  fluorescence of diseased, nonspecificity, 569
  Fusarium stem-end rot, diagnosis, 569
  leaf roll, yield losses by, determination method, 3
  Phytophthora infestans on, control by silver sprays, 17
  purple-top wilt, varietal reactions to, 5
  Rhizoctonia on, control by tuber disinfection, 812
  Rhizoctonia isolates from sugar beet vs., pathogenicity, 49, 274
  ring-spot virus, behavior in roots vs. aerial parts of exp. hosts, 577
  spindling sprout, nature, 340
  tubers, shape, determination method, 184
    shape and development, spindle-tuber diagnostic value, 181
    disinfection by acidulated $HgCl_2$, advantages, 812
  tuber pathogens, Botrytis and Sclerotinia as, 439
  varieties, characterization by tuber dimensions, 184
  viruses, movement and route, 7
  Xylaria tuber rot, 936
  yellow dwarf, virus, "longevity," factors influencing, 3
      purification, 3
      transmission by clover leaf hopper, hereditary variation in, 3
  yields vs. missing plants, determination method, 3
Pratylenchus pratensis, feeding before and during root entry, 862
Presley, John T., 1135
Price, W. C., (21), 756
Proctor, B. E., 201, 213
Protein, virus-, properties in alfalfa mosaic, 394
    quantitative determination technique, 980
Prune, French, diamond canker of, virus etiology (?), symptoms and bud and graft transmission, 886
Prune, mosaic, peach infection (exp.), 355
  Italian-, Sclerotinia spp. on, 726
    vein clearing virosis (new), 464
pruni (Phytomonas), 12
Prunus spp., Armillaria root rot, temp. relations, 3
    buckskin of, distribution, pathology and virus strains, 916
    line-pattern virosis (new) of, distribution relationships and general study, 1004
    peach mosaic on, 323, 326
    Tranzschelia pruni-spinosae discolor from, uredo- and teliospore germination, 189
    vein clearing virosis (new), 463
    virus disease (new) of, 14
  cerasus, virus disease (new) of, 14
  demissa, buckskin disease, 921
    virosis (new) resembling X-disease, 19
  domestica, diamond canker of Agen var., virus etiology (?), symptoms and bud and graft transmission, 886
  mariana, Chalaropsis stem infection (exp.), 859
Prunus virus-7, mottle leaf virosis due to, 467
Prunus virus-8, vein clearing virosis due to, 467
Pryor, Dean E., (534), 863
Pseudococcus comstocki, fungus (new) parasitizing, 5
Pseudomonas group, Phytomonas gardeniae in, 194
Pseudoperonospora spp., sulphur fungicide effects on, 865
  cubensis, on cantaloupe, control by fungicidal dusts, 769
  humuli, on hops, sporulation injury by, 741
Pseudopeziza medicaginis, alfalfa resistance to, 765
  meliloti, sweet clover resistance to, 766

- Psorosis, citrus-, diamond canker of French prune compared, 895
  - heat resistance of virus-A, 861
- **Pteridovirus** n. gen. (viruses), 23
- Puccinia coronata, flexuous hyphal fusions in, 4
  - graminis, flexuous hyphal fusions in, 4
    - teliospores, longevity in laboratory, 197
    - tritici, urediospores, medium for germination, 952
      - on wheat, resistance, inheritance in crosses with Kenya vars., 558
  - helianthi, flexuous hyphal fusions in, 4
  - polysora, on corn, and distribution, 856
    - on teosinte, 856
  - sorghi, on corn, 856
  - suaveolens, on Cirsium arvense, systemic mycelia, urediospore origin, 4
    - heterothallism in, 4
  - triticina, physiologic races in Okla., 5
- Pullularia spp., in chemicallly treated-lumber stain, 272
- Pumpkin, curly top reactions, 860
- Purple-top wilt, potato-, varietal reactions to, 5
- Pythium spp., absence in damping off of longleaf pine, 1012
  - canning-pea root rot due to, control by "Spergon" seed protectant, 20
  - on cotton, 160
  - rhubarb crown rot due to, 863
  - tomato damping-off due to, control in transplants from sand, 2
  - debaryanum, damping-off of broadleaf seedlings by, rotation effects and control, 857
  - **marsipium** n. sp., infection in waterlily leaves, 505
    - morphology, cytology and life history, 492
  - oedochilum, morphology, life history and cytology, 478
  - palingenes, morphology, cytology, and life history, 486

- Quarantines, on fruits and vegetables from Hawaii, 570
- Quercus spp., heartrots, differentiation methods, 6
  - -Hicoria stands, defects in, in Iowa, 99
  - Phytophthora canker, control by tree injection, 12
  - agrifolia, Phytophthora bleeding canker, 863
  - palustris, chlorosis, control by tree injections vs. soil treatments, 18
  - virginiana, Phytophthora bleeding canker, 863
- Quince, Chalaropsis stem infection (exp.), 859

- Rabbits, plant injuries by, control, 23
- radiobacter (Bacillus), 964
- Ramsey, Glen B., 439
- Ramularia tulasnei, Mycosphaerella fragariae as perfect stage of, 239
- Rankin, W. Howard, 19
- "Ratsnest" compost, boron deficient in, 468
- Rawlins, T. E., (864), 916
- Readio, Philip A., (657)
- Red scale, on lemon, Bacillus "C" destroying, 864
- Reports, American Phytopathological Society, annual meeting (1940), 362
  - Pacific Division meeting (1941), 859
  - Southern Division meeting (1941), 767
- Resinous excrescences, on citrus, 861
- Resistance (see also Immunity)
  - alfalfa, to fungus parasites, 765
  - Allium spp., to smut, 9
  - barley, to Ustilago nuda, 351
  - bean, to rust, 26
    - to tobacco mosaic virus, 430
  - cantaloupe, to powdery mildew, 863
  - corn, to Basisporium gallarum, 21
  - cotton, to Fusarium wilt, 771, 1099
    - to Phymatotrichum root rot, 452
    - to root knot, 771, 1099
  - cucurbits, to curly top, 860
  - elms, to Ceratostomella ulmi, 759
    - to Dutch elm disease, 21
  - flax, to Fusarium wilt, 4
  - grasses, to diseases, 1
  - lettuce, to Erysiphe cichoracearum, 534
  - red clover, to fungus parasites, 766
  - rice, to Cercospora leaf spot, 19
  - sugar beet, to curly top, 861
  - sweet clover, to fungus parasites, 766
  - tobacco, to mosaic, 1140
  - tomato, to Fusarium wilt, root knot effects on, 749
  - wheat, to stem rust, 558
- Respiration, plant-cell-, nitrogen starvation effects on, 981
  - tobacco mosaic virus effect on, 25
- Reviews, book-, botany textbook, 677
  - defects in oak-hickory stands, 99
  - diseases of cereal and forage plants in northern U.S.A., 200
  - diseases of vegetable crops, 98
  - insect transmission of plant diseases, 98
  - parasitic plant diseases, 100
  - phytopathology manual, 960
- Rhabditis spp., Harposporium helicoides parasitizing, 795
  - Meria coniospora parasitizing, 793
  - Nematoctonus leiosporus parasitizing, 780
- Rhizoctonia spp., annual stock foot rot due to, 87
  - canning-pea root rot due to, control by "Spergon" as seed protectant, 20
  - in damping-off on longleaf pine, 1011
  - tomato damping-off by, control in transplants from sand, 2
  - solani, comparison of potato and sugar beet isolates, 274
    - on cotton, seedling and boll injury association, 160
      - seedling survival statistics, 1078

damping-off of broadleaf seedlings by, rotation effects and control, 857
on holly, defoliation of cuttings by, and control, 6
mold-toxin effects on, 991
pathogenicity of isolates from potato vs. sugar beet, 49
potato tuber disinfection for, 812
sweet clover root rot due to, distribution, importance and other hosts, 673
rhizogenes (Phytomonas), 964
Rhizoglyphus spp., potato pit-scab role, 9
Rhizosis, date palm Ceratostomella (?), 1123
Rhubarb, Pythium crown rot, 863
Ribes vulgare, white pine blister rust on, relative susceptibility and potential inoculum volume, 732
Rib grass (see Plantago lanceolata)
Rice, Cercospora leaf spot, resistance to, linkage of factors for, 19
Leptosphaeria stem rot, sclerotial longevity, 279
RICHARDS, B. L., 19
Ricinus sp., invading overgrowths in, 15
Rickettsia, as virus genus, 23
Rickettsiaceae, as virus family, 23
RIGLER, NEIL E., (149)
RIKER, A. J., 19, 964
Ring rot, peach virosis, 860
potato bacterial-, 7, 10, 14, 569, 954
Ring spot, potato-, virus behavior in roots vs. aerial parts of exp. hosts, 577
tobacco-, "acquired immunity relations," 533, 1059
effect on pollen and seed set, 527
symptoms in "recovered" plants, 23, 523
virus, activity measurement by local lesion method, accuracy, 21
behavior in roots vs. aerial parts of exp. hosts, 577
distinct strains of, 522
tobacco broad-, virus behavior in roots vs. aerial parts of exp. hosts, 577
RODENHISER, H. A., 175
Rodents, injurious, control, current research, 23
Root diseases, pine-, 25, 803, 1063
Root knot (see Heterodera marioni)
Root rots (see also Rots)
Armillaria-induced, 3
barnyard grass-, 832
citrus Armillaria, 859
Clytocybe, of pear and tung trees, 93
cotton Phymatotrichum, 358, 452
pea-, 20
sugar beet Rhizoctonia, 49
sweet clover Rhizoctonia, 673
tobacco Thielaviopsis black-, 6
wheat-, 379
Roots, nematode feeding before and during entry of, 862
nematodes parasitizing aerial parts vs., morphological comparisons, 645
pathogens entering via, solution-culture infection methods for, 549
virus behavior in aerial parts vs., 575
virus concentration in extracts of, 591
viruses in extracts of, thermal inactivation, 594
viruses inoculated into, factors influencing movement, 589
Rootstocks, rodent damage to, control, 23
Rosa spp., Chalaropsis stem infection (exp.), 859
Rose, Chalaropsis-induced bud-union failures, 859
powdery mildew, control as influenced by wetting agents in greenhouse, 16
ROSS, A. FRANK, 394, 410
Rotations, crop-, effect on damping off of broadleaf seedlings, 857
for tobacco bacterial Granville wilt control, 20
ROTH, ELMER R., (6)
Rots (see also Root rots; Heartrots)
Barbarea vulgaris black-, 347
barnyard grass basal stem-, 832
celery Cercospora greenish black-, 17
cherry Sclerotinia spp. brown-, 469
cordwood-, 22
corn Basisporium cob-, 21
cotton boll-, anthracnose and angular leaf spot organisms associated in, 24
fungi associated, 158, 769
cotton southern sclerotial, 944
crucifer bacterial black-, 347
Fomes fomentarius wood-, 11
grape (muscadine) black, 767
grape (muscadine) Macrophoma soft-, 768
grape (muscadine) Melanconium bitter-, 768
lupine Fusarium hypocotyl-, 771
potato bacterial ring-, 7, 10, 14, 569, 954
on tomato, 14
potato Fusarium stem-end-, 569
potato tuber-, Botrytis and Sclerotinia causing, 439
potato Xylaria tuber-, 936
rhubarb Pythium crown-, 863
rice Leptosphaeria stem-, 279
stone-fruit Sclerotinia brown-, 718
sugar cane red-, 767
tomato Phomopsis, 24
Rotylenchus erythinae, feeding before and during root entry, 862
reniformis, on cotton, distribution in regional wilt plats, 771
feeding before and during root entry, 862
RUEHLE, GEO. D., 95, 936, 947
Rugose mosaic, potato-, virus movement and route, 7
RUMBOLD, CAROLINE T., 19
Russula fragilis, mycorhizal, on pine, 568
Rusts, bean-, 26
of Cirsium arvense, Puccinia suaveolens causing, 4
corn-, 856
flax-, 852
on Prunus spp., Tranzschelia prunispinosae discolor-induced, 189

  wheat leaf-, 5
  wheat stem-, 197, 558, 952
  white clover Uromyces leaf-, 554
  white pine blister- (see Cronartium ribicola)
Rye, stem smut, infection and development factors and control, 617
RYKER, T. C., 19

Salix spp., blight of, Physalospora vs. Fusicladium as cause in Canada, 1056
Saltation, in Venturia inaequalis, 1142
  in Verticillium albo-atrum, 1135
Salvia splendens, tobacco ring-spot inoculations, 580
SAMPSON, H. C., (677)
Sapwood rot, by Fomes fomentarius, 11
savastanoi (Bacterium), 361
  (Phytomonas), 361
Scab, apple Venturia, 7, 13, 296, 1142
  poinsettia Sphaceloma, 947
  potato Actinomyces, 702, 954
  potato Actinomyces pit-, 9
scabies (Actinomyces), 9, 702, 954
Scale, red-, on citrus, Bacillus "C" destroying, 864
Sclerospora macrospora, on oats in Miss., 768
Sclerotia, of Leptosphaeria salvinii, longevity, 279
Sclerotinia spp., as potato tuber pathogens, 439
  americana, mold toxin effects on, 1002
  fructicola, apothecia, factors influencing development, 1023
    conidial respiration, 9
    hosts, host relations and cross inoculations in W. Washington, 718
    on sweet cherry, incidence in Ore., 469
  laxa, hosts, host relations and cross inoculations in W. Washington, 718
    on sweet cherry, incidence in Ore., 469
Sclerotium spp., on cotton, 160
  rolfsii, on cotton in Arizona, 944
    sugar beet infection, 946
Scolytus multistriatus, as vector of Dutch elm disease, 19, 657
SCOTT, C. EMLEN, (14)
Sedum sp., crown gall on, temp. relations, 976
Seed transmission of: Cercospora sesami, 770
  cowpea mosaic, 424
Seed treatment, benne, for Cercospora blight, 770
  cereal, by Oklahoma hot-water seed treater, custom operation, 5
  cotton-, germination vs. storage, 768
    by organic mercurials, 768
    for seedling diseases, 1078
    tests in 1940, 769
  for pea root rot, by "Spergon," 20
  potato, for bacterial ring rot, 7, 954
  wheat, for root rots, 379
    for seed-borne diseases, 392
Seedling diseases, cotton-, 1078
Selenophoma bromigena, spore germination, types and factors influencing, 953
SEMENIUK, G., 20
sepedonica (Phytomonas), 7, 10, 14, 954
sepedonicum (Bacterium), 569
Serological tests, of crown gall, hairy root and radiobacter organisms, 968
Septoria musiva, Mycosphaerella populorum as perfect stage, 241
  populicola, Mycosphaerella p. as perfect stage, 241
Sesamum indicum, Cercospora blight, control by hot water seed treatment, 770
Severe etch, virus behavior in roots vs. aerial parts of exp. hosts, 577
SHANOR, L., (1)
SHAPOVALOV, MICHAEL, 864
SHARVELLE, E. G., 20
SHAW, K. J., 21, (6)
SHAW, LUTHER, 770
SHEMA, B. F., (20)
SHERBAKOFF, C. D., 770
Shot-hole, celery Marssonia, 17
SHOWALTER, J. W., (10)
SILBERSCHMIDT, K., 430
Silktree (see Albizzia julibrissin)
Silver nitrate, liquid conduction route in wood shown by, 1035
Silver sprays, fungicidal and adhesive properties, 17
Single-spore cultures, terminology, 471
"Single-spored," "monosporous" vs., 471
Sinningia speciosa, boron deficiency disease of, cause, description and control, 467
SMITH, A. L., 771, 1099
SMITH, CLAYTON O., (189), 361
SMITH, RALPH E., 886
SMITH, T. E., 20, 21
SMITH, W. K., (765)
SMUCKER, S. J., 21, 758
Smut(s), of Allium spp., 9
  barley loose-, 22, 284, 351
  barley covered-, 175
  cereal loose-, 5
  corn Sorosporium, on male inflorescence, 1046
  corn Ustilago, 333, 1051
    on male inflorescence, 1046
  grass-, sori from ovarial and staminal tissues, variations in, 1043
    variations in cultures of, 761
  rye stem-, 617
  sorghum covered kernel-, 254
  wheat loose-, 24
SNELL, WALTER H., 475, 732
Snowmold, of grasses, basidiomycete causing, 1058
  turf-, eastern form, Typhula itoana causing, 940
SNYDER, W. C., 1054
Sodium dinitro-cresol, crown gall on almonds controlled by, 956
Sodium nitrate, effect on Fusarium oxysporum cubense, 564
Soil, Fusarium oxysporum var. cubense growth in, 91
Soil amendments, effect on apothecial development in Sclerotinia, 1023
Soil fumigants, for rodent control, 23
Soil treatments, for chlorosis of pin oaks, 18

  in tobacco plant-beds, 6
SOKOLOFF, V. P., 864
solanacearum (Bacterium), 20
Solanum spp., virus inoculations, 579, 580
Sorghum, covered kernel smut, heritable lysis in germinating chlamydospores, 254
  Phoma terrestris infecting (exp.), 913
  Sphacelotheca smut sori on staminal tissues, diagnostic value, 1043
Sorghum halipense, Sphacelotheca sori on ovarial tissues, diagnostic value, 1043
Sorosporium everhartii, on Andropogon furcatus, sori on ovarial and staminal tissues, diagnostic value, 1043
  reilianum, on corn male inflorescence, 1046
  syntherismae, cultural variations in, 762
Soybean, Phoma terrestris isolated from, 914
  Rhizoctonia root rot, 674
Specimens, demonstration-, storage and mounting technique, 763
SPENCER, ERNEST L., 21
''Spergon,'' as seed protectant for canning peas, 20
Sphaceloma sp., poinsettia scab due to, pathology, 947
Sphacelotheca cruenta, on sorghum, sori on staminal and ovarial tissues, diagnostic value, 1043
    on Sorghum halepense, sori on ovarial tissues, diagnostic value, 1043
  occidentalis, on Andropogon furcatus, sori on ovarial tissues, diagnostic value, 1043
  sorghi, chlamydospores, heritable lysis in germinating, 254
    on sorghum, sori on staminal and ovarial tissues, diagnostic value, 1043
    solopathogenicity vs. lethal crosses, 261
  panici-miliacei, cultural variations in, 762
Sphaerotheca spp., conidia, substances stimulatory to, 865
    on rose, control as influenced by wetting agents in greenhouse, 16
Spicaria **coccospora** n. sp., nematodes parasitized by, 787
Spinach, downy mildew, sporulation injury by, 741
  Phoma terrestris infecting (exp.), 913
  virus inoculations, 579, 580
Spindle tuber, potato-, diagnosis by shape and development of tubers, 181
Spindling sprout, potato-, nature, 340
Spore-germination tests, of fungicides, duration of inhibition in, 24
Spores, Ceratostomella, microagglutination, 15
  fungus-, toxicity ratings of compounds by, 16
Spot, pepper and tomato bacterial-, 753
  tomato bacterial-, 25, 753
Sprays, coverage index vs. inoculum potential, measurement technique and relations, 7
  silver-, fungicidal and adhesive properties, 17
Squash, China-, curly top reactions of, 860
  Phoma terrestris infecting (exp.), 913
Squash mosaic, virus, insects transmitting, and properties, 8
Stagonospora meliloti, alfalfa resistance to, 766
    sweet clover resistance to, 766
Stain fungi, wood- (see under Wood staining)
Stain technique, for liquid conduction route in wood, 1035
Stalk rots, corn-, 20
STANDEN, J. H., 21
STANDRING, ELIZABETH T., (15)
STEINER, HANS, 867
STEINMETZ, F. H., 22
Stem spot, pentstemon Ascochytella, 194
Stemphylium botryosum, alfalfa resistance to, 766
  sarcinaeforme, red clover resistance to, 766
Stems, nematodes parasitizing roots vs., morphological comparisons, 645
  root knot parasitism in, 634
Stereum murrayi, in culture, 85
    hardwood heartrot and canker association, 82
Sterilization, of organic compounds used in culture solutions, methods quantitatively compared, 149
stewartii (Phytomonas), 7
Stock, annual- (see Matthiola incana)
STONE, G. M., (6)
Stone fruits, Armillaria root rot, temp. relations, 3
  brown rot, Slecrotinia spp. and host relations in W. Washington, 718
  buckskin of, distribution, pathology, and virus strains, 916
    reactions of different hosts to virus strains, 864
  line pattern virosis (new) of, distribution, relationships and general study, 1004
  peach ring rot grafted into various, 860
  Sclerotinia brown rot, 9
    apothecium development factors, 1023
  Tranzschelia pruni-spinosae discolor from, uredo- and teliospore germination, 189
  vein clearing virosis (new), 463
Strawberry, crinkle plus stunt infection, 850
  everbearing-, yellowing in, genetic origin, 1
  Mycosphaerella purple leaf spot (new), distribution and general study, 225
  stunt, virus-induced (new), 849
Strawflower (see Helichrysum bracteatum)
Streak, tobacco-, virus behavior in roots vs. aerial parts of exp. hosts, 577
Stunt disease, strawberry-, new virus causing, 849
Sugar beet, curly top, resistance vs. host age, 861

  virus, leaf hopper vector relations to strains, 8
    strain combinations, 8
    transmission via dodder, 13, 652
  dodder relation to healthy vs. curly top-infected, 861
  Rhizoctonia isolates from potato vs., pathogenicity, 49, 274
  southern sclerotial rot, 946
Sugar cane, Myriogenospora infection and distribution, 564
  red rot, incidence vs. spore load, 767
SULLIVAN, J. T., 554
Sulphur deficiency, in cotton, symptoms, 771
Sunflower, crown gall of, secondary tumor and strand development in, 135
  Verticillium albo-atrum infection (exp.), 1134
Supplements, to copper fungicides, effect on solubility, determination method, 17
Susceptibility, corn, to bacterial wilt, 7
  oats, to smuts, basis of differing reactions, 673
Sweet clover, resistance to fungus parasites, 766
  Rhizoctonia root rot, distribution and importance, 673
Sweet potato, Fusarium wilt, and cross inoculations, 1, 21
Sweet william, Fusarium wilt (new), 1054
SWINGLE, R. U., 22
Sycamore (see Platanus)
Systremma **acicola** n. comb., pine brown spot needle disease due to, distribution and general study, 70

tabacum (Bacterium), 6, 957
TAKAHASHI, WILLIAM N., (862), 864, 1117
TALLEY, PAUL J., (926)
TAPKE, V. F., 22, 284
TAYLOR, A. L., (771)
TAYLOR, CARLTON F., 22, (1047)
Technique, air-borne microorganisms, etc., appraisal, 201
  chromoprotein quantitative determination, 980
  crop yields vs. missing plants, determination, 3
  culture, single-spore, terminology, 471
  culture media, for Actinomyces scabies, 714
    downy mildew conidial stimulation by, 865
    for Puccinia urediospore germination, 952
    sterilizing organic compounds for, methods quantitatively compared 149
    for Verticillium albo-atrum, 1135
  diagnosis, Colletotrichum latent infections, by methyl bromide, 570
    Cronartium spp., pH method vs. temp., 1
    of loose smuts of barley, 284
    potato bacterial ring rot, by fluorescence, 10, 569
    of wood-rotting fungi, 6
  experimental, disease data in factorial designs, 26
  fungicides, assay tests, hanging-drop method of applying, 1109
      spore-germination method, 1108
    protective value, laboratory prediction, 12
    solubility as influenced by supplements, determination method, 17
    spore-germination tests, 24
    sulphur, effects on downy mildews, evaluation, 865
    testing, in liquid culture, 149
  growth rate determination in fungi, photoelectric method, 448
  infection mechanism, micrugical study method, 11
  inoculation, for Armillaria mellea, 859
    inoculum preparation by mechanical liquefier, 566
    for Phymatotrichum omnivorum in sand culture, 358
    solution-culture methods for Fusarium wilts, 549
    for Ustilago nuda, 351
  potato tuber shape determination, 184
  selection for resistance, cotton to root rot, 452
  specimens for demonstration, storage and mounting, 763
  staining, for liquid conduction route in wood, 1035
  sterilization of organic compounds for use in culture solutions, methods quantitatively compared, 149
  toxicity ratings of compounds, fungus method, 16
  viruses, activity measurement, local lesion method, accuracy, 21
    extraction, 411
    inactivation and reactivation by chemicals, mechanism, 681
    indicator plant method, 430
    quantitative measurements of, 12
    study through dodder union of hosts, 13
TEMPLE, LAWRENCE, (5)
Teosinte, Puccinia polysora on, 856
Terminology, ''single-spored'' vs. ''monosporous,'' 471
TERVET, IAN W., 672
Thallovirus n. gen. (viruses), 23
THARP, W. H., 26, 771
Thermal death point, fungus-, genetic role, 264
  growing conditions vs., 264
Thermal inactivation, of viruses in root extracts, 594
Thiamin, effect on Erysiphe cichoracearum, 863
Thielaviopsis basicola, in tobacco plant-beds, soil treatments for, 6
THOMAS, H. EARLE, (916), (859), 864
THOMAS, W. D., JR., 567
THOMPSON, G. E., 241
THORNBERRY, H. H., 23
THURSTON, H. W., JR., 1047

Tiffany, L. H., (677)
Tilford, P. E., (22)
Tilletia levis, hybridization with T. tritici, 80
tritici, dwarf bunt form, on fall-sown spring wheat, 951
germination tests, culture, pathogenicity and hybridization, 74
Timber (see Lumber; Wood)
Tims, E. C., 771
Tipburn, cabbage-, potash effects on, 18
Tippett, R. L., (564)
Tobacco, alfalfa mosaic inoculation, virus concentration at intervals after, 410
blackfire, epidemiology, light vs. water-soaking in, 957
bacterial Granville wilt, control by crop rotations, 20
broad ring spot, virus behavior in roots vs. aerial parts of exp. hosts, 577
Cercospora frog eye, conidial production in culture, 97
dodder-histological relation to healthy vs. curly top-infected, 861
etch virus, quantitative measurements of, 12
Fusarium wilt, absence following cotton and cowpea, 21
and cross inoculations, 1, 21
mosaic, changes in N and virus content of detached leaves in darkness, 1117
inclusion bodies, leaf-starvation effects on, 1121
resistance to, Ambalema vs. glutinosa types, significance, 1140
transmission via dodder, 13
virus, Brazilian bean vars. as indicators, 430
chemical inactivation and reactivation, mechanism, 679
effect on cellular oxidation, 25
inactivation, nitrogen relations, 21
by X-rays vs. pH, 862
inactivator from yeast, 864
multiplication, host growth vs., 861
P-nutrition vs., 861
new var. from rib grass, 1089
-protein, synthesis vs. leaf chromoprotein and cell metabolism, 978
starvation effects on stream double refraction, 1121
transmission via dodder, 653
virus-1, behavior in roots vs. aerial parts of exp. hosts, 577
Peronospora downy mildew, control by copper-soap spray, 286
plant-beds, soil treatments for weeds and diseases, 6
ring spot, acquired immunity relations, 533, 1059
effect on pollen and seed set, 527
symptoms in "recovered" plants, 23, 523
virus, behavior in roots vs. aerial parts of exp. hosts, 577
distinct strains of, 522
streak, virus behavior in roots vs. aerial parts of exp. hosts, 577
viruses, activity measurement, local lesion method, accuracy, 21
wildfire, epidemiology, light vs. water-soaking in, 957
mechanism of invasion, 6
Tomato, Alternaria early blight, copper spray tests for, 25
sprays for, timing and dosage, 11
bacterial spot, copper spray tests for, 25
variations in, 753
bushy stunt, transmission via dodder, 13, 652
copper fungicides tested on, 10
crown gall on, host-parasite relations, 972
infection mechanism, micrugical study, 11
temp. relations, 975
curly top on, plant populations in relation to, 864
damping-off, control in transplants from sand, 2
Fusarium wilt, epinasty as early symptom, 281
root knot effect on, 749
variations in single-spore isolates of strains, 103
lightning injury, symptomatology, 1017
Phoma terrestris infecting (exp.), 914
Phomopsis fruit rot, 24
phyllody in vars., anatomy and morphology, 168
potato bacterial ring-rot infection (exp.), 14
root knot parasitism in leaves and stems, 635
Verticillium albo-atrum infection (exp.), 1134
virus inoculations, 579, 580
yields as influenced by copper sprays, 25
Tompkins, C. M., (467), 1130
Toole, E. Richard, 599
Toxicities, of compounds, to fungi, technique of measurement, 24
ratings by different fungi, 16
Toxicity diseases, citrus-, 860
Transeau, E. N., 677
Transpiration, leaf-, vapor pressure gradient above, 1040
Tranzschelia pruni-spinosae discolor, uredo- and teliospore germination and factors influencing, 189
Trees (see also Lumber; Wood)
broadleaf-, damping-off, forms and rotation effects, 857
coniferous, mycorhizas of, in Germany, 567
hardwood-, defects in oak-hickory stands in Iowa, 99
heartrots, differentiation methods, 6
Phytophthora canker, control by tree injection, 12
Stereum murrayi-associated heartrot and cankers, 82
Trees, injections for pin oak chlorosis, 18
Trees, orchard fruit- (see also Fruits; Stone fruits)
rodent damage to, control, 23

Trichoderma spp., in damping off of longleaf pine, 1012
  toxin of, production and extraction, 992
Trifolium spp., Rhizoctonia root rot, 674
  hybridum, basidiomycete killing, 1058
  pratense, basidiomycete killing, 1058
    resistance to fungus parasites, 766
  repens, carotene in leaves as influenced by sulphur treatment, 556
    mosaic, transmission via dodder, 13
    Uromyces leaf rust, effect on carotene content, 554
    White Dutch var., basidiomycete killing, 1058
Truck crops, diseases, book review, 98
Tuberculariaceae, gardenia leaf spot due to fungus of, 2
Tubers, rodent damage to, control, 23
Tucker, C. M., 844
Tulip, virus infections, current-season symptom development, production of, 838
    methods compared, 838
Tullis, E. C., 279
tumefaciens (Bacterium), 15
  (Phytomonas), 11, 19, 135, 956, 964
Tumors (see also Crown gall; Overgrowths)
  Phomopsis-induced, in elm and maple, 544
Tung tree, Clitocybe-associated root rot, 93
Turf, eastern snowmold of, Typhula itoana causing, 940
  fungus disease (new), Helminthosporium or Curvularia causing, 24
Tuthill, C. S., (3)
Tyler, Leon J., (657)
Typhula itoana, eastern snowmold of grasses due to, 940

Ullstrup, Arnold J., (158), 508
Ulmus spp., Dutch elm disease, fungus, in bark beetle-infested wood, 19
      microagglutination of fungus spores, 15
      transmission by European elm bark beetle, 657
    pathogenesis, 660
    reactions compared, 21, 758
  Phytophthora bleeding canker, 863
    control by tree injection, 12
  americana, Dutch elm disease, vascular invasion vs. vessel length, 2
    mosaic, new mottled-leaf-, and distribution, 22
    Phomopsis tumor of, 544
  pumila, Chalaropsis root infection (exp.), 859
Ultraviolet light, potato bacterial ring rot detection by fluorescence under, 10, 569
Uncinula spp., conidia, substances stimulating, 865
Urediospores, cereal rust-, germination, medium for, 952
Urocystis cepulae, on Allium spp., resistance, 9
  occulta, cross inoculations on wheat negative, 628
    rye stem smut due to, infection, development factors and control, 617
Uromyces spp., flexuous hyphae in, 4
  appendiculatus, on bean, resistance to physiologic races, 26
  trifolii, red clover resistance to, 766
    trifolii-repentis, on white clover, effect on carotene content, 554
Ustilago avenae, on oats, susceptibility of seed lots differing, 673
  crameri, cultural variations in, 762
  hordei, chlamydospores, germination, X-ray effects, 175
    X-ray effects on, 175
  levis, on oats, susceptibility of seed lots differing, 672
  medians, untenability of sp., 22
  neglecta, cultural variations in, 762
  nigra, on barley, identification technique, 284
    differentiation and relationships, 22
  nuda, on barley, seed treatment by Oklahoma hot-water seed treater, 5
    differentiation and relationships, 22
    on fall-sown barley, resistance of vars. to, 351
    inoculation technique, 351
  sphaerogena, cultural variations in, 762
  tritici, on wheat, seed treatment by Oklahoma hot-water seed treater, 5
      varietal reactions to, 24
  zeae, chlamydospores, germination variations, 333
      inheritance of germination type in, 339
    on corn male inflorescence, 1046
    pathogenicity of paired haploid lines vs. numerous mixed haploids, 1051

Valleau, W. D., 6, 23, (97), 357, 522
Vapor pressure, gradient above transpiring leaves, 1040
Variegation, bean leaf-, inheritance, 26
Vectors, insects as (see under Insects)
Vegetables, diseases, book review, 98
  quarantines on, from Hawaii, 570
Vein clearing, stone-fruit virosis (new), 463
Venturia inaequalis, on apple, epidemiology, 297
    eradicant fungicides for, 296
    ground sprays for, 13
    pathogenicity, genetic relations, 1142
    spray coverage index vs. inoculum potential, measurement and relations, 7
  saltation in, nature, 1142
Verrall, A. F., 270
Verticillium albo-atrum, saltants of, medium for production, 1135
    from single-spore culture, and taxonomic significance, 1135
  strawflower wilt due to, general study and other hosts, 1130
  taxonomic history, 1135, 1138

vesicatoria (Phytomonas), 753
vesicatorium (Bacterium), 25
Vicia angustifolia, Rhizoctonia root rot, 674
  faba, virus infections, isomeric crystals in, 760
Viruses (plant—special and general data)
  acquired immunity, competition vs., 23
    disease phases vs., 533
    general discussion, 1059
    in mosaics, 1059
    use of term, 1061
  activity, local lesion measurement method, accuracy, 21
  antagonisms among, 1059
  behavior in roots vs. aerial parts, 575
  changes in N and virus content of detached leaves in darkness, 1117
  effect on cellular oxidation, 25
  extraction technique, 411
  heat therapy, in aster yellows, 14
  inactivation, destruction vs., 699
    nitrogen nutrition effects, 21
    in vivo, 415, 419
    reactivation, and mechanism, 700
      technique of chemical, 681
  inactivator from yeast for, 864
  indicator plant method, for tobacco mosaic, 430
  infection as influenced by nutrients, 10
  inoculation methods compared, in tulip, 838
  insect transmission, hereditary variation in, 3
  isomeric crystals in Pisum virus-2 and Phaseolus virus-2, 760
  movement, in inoculated roots, factors influencing, 589
    in plant parts, direction and rate, 575
    route and, 7
  multiplication, vs. P-nutrition and host growth in tobacco mosaic, 861
    in vector (aster yellows), 126
  nature and origin, 989
  nomenclature, and classification, new system, 23
    system suggested for citrus-, 356
  nucleoprotein of, properties in alfalfa mosaic, 394
  plant disease-, book review, 100
  protein of, quantitative determination technique, 980
  quantitative measurements of, 12
  "recovery" from infection vs. disease phases, 533
  in root extracts, concentration, 591
    thermal inactivation, 594
  severe etch, 577
  study through dodder union of hosts, 13
  symptomatology as influenced by concentration, 418
  synthesis of protein of, relation to leaf chromoprotein and cell metabolism, 978
  transmission via dodder, 13, 649
  vector transfer of strains simultaneously, 8
  X-ray inactivation as influenced by pH, in tobacco mosaic, 862
Viruses (by plant hosts—for detailed data see under hosts and specific diseases)
  alfalfa, mosaic, 21, 394, 410
  aster, yellows, 13, 14, 124, 651
  bean, mosaic, 10, 577
      leaf variegation (heritable) resembling, 26
  cherry (sour), yellows, 355
  citrus, nomenclature system suggested, 356
    psorosis, strain-A, 861
  commelina, mosaic, 756
  cowpea, mosaic, 420
  cucumber, mosaic, 577, 756
  cucurbits, mosaic, 8, 13, 652
  cushaw, curly-top reactions in, 860
  elm, mosaic (new), 22
  fig, mosaic, 561
  grape, Pierce's vine disease (?), 862
  Nicotiana glutinosa shoot enations (?), 2
  peach, mosaic, 4, 322
    mottle, 860
    ring-rot, 860
    rosette-mosaic, 353
    wart, 859
    X-disease-like, 19, 860
  Phaseolus-2, 760
  Pisum-2, 760
  Plantago spp., mosaic, 1089
  potato, 7
    blue-stem (?), 5
    leaf roll, 3
    purple-top wilt (?), 5
    ring spot, 577
    spindle tuber, 181
    yellow dwarf, 3
  prune, diamond canker of French (?), 886
    mosaic, 355
  of Prunus spp., new, 14, 19, 463, 886, 1004
    vein clearing, 463
  pumpkin, curly-top reactions in, 860
  of stone fruits, 1004
    buckskin, 916, 864
    line-pattern virosis, 1004
  strawberry, crinkle, 850
    stunt, 849
  sugar beet, curly top, 8, 13, 652, 861
  tobacco, broad ring spot, 577
    etch, 12
    mosaic, 13, 21, 25, 430, 577, 653, 679, 861, 862, 864, 978, 1089, 1117, 1140
    necrosis, 21
    ring spot, 21, 23, 522, 577
    streak, 577
  tomato bushy stunt, 13, 652
    curly top on, 864
  tulip, 838
  white clover, mosaic, 13
      virus-1, 654
Vitamin B_1, effect on Erysiphe cichoracearum, 863

WADLEIGH, C. H., (26), (771)
WALKER, J. C., (14), 23
WALKER, JOHN CHARLES, 98
WALTER, JAMES M., 23, (349)
Wart, peach-, virus-induced, 859
Water, loss from inert surfaces, Bordeaux effects on, 865

WATERMAN, ALMA M., (475)
WATSON, I. A., 558
WEAVER, L. E., (849)
Weeds, in tobacco plant-beds, soil treatments for, 6
Weevils, barnyard grass root rot and basal stem rot relations to, 832
WEIGLE, C. G., 865
WEIMER, J. L., 771, 1031
WEINDLING, RICHARD, 24, 158, (769), 991
WEISS, FREEMAN, 471
WELLMAN, FREDERICK L., 103, 281
WELLMAN, R. H., (16), 24
WELLS, W. F., 201, 203
WERNHAM, C. C., 24, 940
Wetting agents, effect on rose powdery control in greenhouse, 16
Wheat, disease-free seed, mercurial dust effects on, 387
  dwarf bunt, on fall-sown spring-, 951
    Tilletia tritici race causing, 74
  leaf rust, physiologic races in Okla., 5
  loose smut, seed treatment by Oklahoma hot-water seed treater, custom operation, 5
    varietal reactions to, 24
  Phoma terrestris infecting (exp.), 913
  root rots, seed treatments by mercurial dusts, 379
  seed-borne diseases, seed treatments by mercurial dusts, 392
  stem rust, resistance inheritance in crosses with Kenya vars., 558
    teliospore longevity, 197
    urediospore germination, medium for, 952
  winter-, basidiomycete killing, 1058
  yields as influenced by seed disinfection and soil fertilization, 385
WHIPPLE, OTIS C., 1017
WHITAKER, THOMAS W., 534, (863)
White clover, mosaic, virus-1, transmission via dodder, 654
White pine blister rust (see Cronartium ribicola)
WIANT, JAMES S., 24
WILCOXON, FRANK, (16)
Wildfire, tobacco bacterial-, 6, 957
Willow, blight, Physalospora vs. Fusicladium as cause in Canada, 1056
Wilsonema spp., Harposporium oxycoracum parasitizing, 797
    Nematoctonus leiosporus parasitizing, 780
Wilt(s), banana Panama-, 564
  corn bacterial-, 7
  cotton Fusarium, 770, 771
  flax Fusarium, 4
  Fusarium forms and hosts, 1
  mimosa tree Fusarium, 599
  root-infecting types, solution-culture inoculation methods, 549
  strawflower Verticillium, 1130
  sweet potato Fusarium, 21
  sweet william Fusarium (new), 1054
  tobacco bacterial Granville-, 20
  tobacco Fusarium, 21
  tomato Fusarium, 103, 281, 749
WINGARD, S. A., (10), 24
Wintercress, bitter-, bacterial black rot (new), 347
WODEHOUSE, R. P., 201, 206
WOLF, FREDERICK A., 61
Wollbachia, as virus genus, 23
Wood (see also Lumber; Trees)
  blue-stain fungi of, ascospores, 19
  cord-, decayed, chemical composition and calorific value, 22
  Fomes fomentarius rotting, 11
  fungi inhabiting, growth rates compared, 825
  fungus rots, differentiation methods, 6
  liquid conduction in, as influenced by larval gallery scars, determination, 1035
    silver nitrate stain showing route, 1035
  staining in chemically treated lumber, fungi associated, 270
WOODS, MARK W., 25, 677, 978
Worms, Annelid-, potato pit-scab role, 9
Wounds, dressings for, 23
WRIGHT, ERNEST, 857

Xanthomonas **barbareae** n. sp., Barbarea vulgaris black rot due to, 348
X-disease, peach-, new virosis resembling, 19, 860
X-rays, effect on Ustilago hordei, 175
  tobacco mosaic virus inactivation by, pH effects on, 862
Xylaria apiculata, morphology, 938
    potato tuber rot due to, 939

YANG, JUHWA Y., (664)
YARWOOD, C. E., 741, 865
Yeast, virus inactivator from, 864
Yellow dwarf, potato-, virus, longevity, factors influencing, 3
    purification, 3
    transmission by clover leaf hopper, hereditary variation in, 3
Yellowing defect, in strawberry, genetic origin, 1
Yellows, aster-, heat therapy, 14
    virus, incubation and multiplication in vector, 126
      mechanical transmission between leaf hoppers, 124
      transmission via dodder, 13, 651
  cherry (sour)-, history, distribution and virus etiology, 355
Yields, crop-, missing plants vs., determination method, 3
YORK, HARLAN H., 25
YOUNG, P. A., 25, (749)
YU, EMERSON H., (264)

ZAUMEYER, W. J., 26
ZELLER, S. M., 463, 849
ZEMLANSKY, JOHN, (15)
ZENTMYER, GEORGE A., 896
Zinc deficiency, in citrus, 861
Zinnia elegans, commelina mosaic virus infecting (exp.), 756
    virus inoculations, 579, 580
Zooviraceae new fam. (viruses), 3
Zoovirus n. gen. (viruses), 3

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(Note: The arrangement of the abstracts is alphabetical by author or senior author.)

The Problem of Controlling Certain Diseases of Forage Grasses by Selection and Hybridization. Aamodt, O. S., C. L. Lefebvre, and H. W. Johnson. The increased attention paid to pasture and meadow grasses in the past few years has revealed several diseases that present a definite handicap to the effective use of these grasses in certain localities. Sudan grass, of extreme value in dry regions, is almost worthless in the Eastern humid portion of the United States because of the ravages of several foliage diseases. Dallas grass, a valuable grazing plant in the Southeast, is extremely susceptible to ergot (*Claviceps paspali* S. & H.), and this disease is one of the chief factors limiting the production of Dallas grass seed. Kentucky bluegrass, one of the most important pasture grasses, is subject to attack over widespread areas each season by a leaf spot (*Helminthosporium vagans*). The production of disease-resistant strains of these grasses by selection and hybridization appears to be the only logical control measure. Some of the progress being made in this direction at several places by workers in the Division of Forage Crops and Diseases and State Experiment Stations will be demonstrated.

Temperature as a Precision Factor in Chemically Distinguishing Cronartium ribicola and C. occidentale. Acree, Ruby J. A microchemical colorimetric pH procedure for differentiating the telia of *Cronartium ribicola* and *C. occidentale* was published in 1937 and has since been in constant use for identification of these two rusts in connection with the California eradication program. During this period the operations have been carefully studied for the purpose of detecting any difficulties or limitations in the method, as published, that would result in erroneous identification. When followed exactly, the procedure gave excellent results with the exception of two periods, one in October, 1938, and the other in July, 1940. After eliminating all other sources of error, temperature effect was considered. The working conditions provided 60° F. in one period and the upper nineties in the other. In both cases changing to the seventies gave satisfactory results. For confirmation of these results a short series of tests was run, which showed that temperatures below 70° and above 90° F. were not satisfactory for the best results. As evidence indicates that the success of this procedure is probably due to a difference in permeability of the telia of the two species and as it is well established that temperature is a controlling factor in pH activity, it is quite logical to suppose that such a procedure would not give satisfactory results over too wide a temperature range.

Yellowing in Everbearing Progressive Strawberries. Afanasiev, M. M., and H. E. Morris. Progressive strawberries in Montana manifest an interveinal yellowing of their leaves, usually distributed uniformly throughout the leaf blade. Sometimes, when its intensity is not so distributed, it appears as a marginal yellowing or as a mottling. One or more of these modifications of yellowing may be present on the same plant. The plants do not retain the same type of yellowing throughout the season. Leaves of plants showing severe stages very often are disfigured and twisted along the midrib. These plants usually bcome dwarfed and die. Yellowing is most pronounced in early spring and gradually decreases toward fall. It is transmitted through seeds and runners. All attempts to transmit the disease from diseased to healthy plants by grafts or by the insect (*Myzus fragaefolii*) have been negative. Seeds of 5 plants that remained healthy for several years were germinated. The resulting plants did not yellow. They were increased by runner production and at present there are several hundred Progressive seedlings free from yellowing. The results of this investigation indicate that the yellowing is genetical in character.

Some Fusarium Wilt Organisms. Armstrong, G. M.,[1] L. Shanor,[1] C. C. Bennett,[1] and B. S. Hawkins.[2] Inoculations with Fusarium-wilt organisms from the following plants were made in soil and in water culture on cotton, sweet potato, okra, cowpea, tobacco, mimosa (*Albizzia julibrissin*) and coffee weed (*Cassia tora*). Records were made of external symptoms of wilt, internal blackening, and recovery of the fungus by plating. The cotton fungus caused definite wilting in tobacco, okra, and coffee weed. This fungus was recovered from 49 per cent of sweet potato plants, but only 3 per cent showed exter-

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[2] Division of Cotton and Other Fiber Crops and Diseases, U.S.D.A.

nal symptoms of wilt. Cotton and tobacco inoculated with the sweet-potato fungus showed little external wilting, but the fungus was recovered from 32 and 55 per cent of the plants, respectively. The okra fungus caused definite wilting of cotton and tobacco. The coffee-weed fungus caused wilt of cotton and tobacco. Cotton, coffee weed, and tobacco were unaffected by the mimosa fungus. Cross inoculations of cotton and cowpea were unsuccessful. Isolates of the cotton-wilt fungus from plants growing a few rows apart may be highly pathogenic or relatively weak. Isolates from a field where resistant varieties are usually killed were no more pathogenic than those from another field where resistant varieties usually survive. Isolates from susceptible and resistant varieties were equally pathogenic.

Relation of Vessel Length at Infection Points to Extent of Vascular Invasion in American Elm by Ceratostomella ulmi. BANFIELD, W. M. Vessel length at various points in American elm stems was determined in June by injecting spores of *Ceratostomella ulmi* 1 hour before felling the trees and, later, examining for vascular discoloration induced by the injected spores. Vessels found in new shoots were only a few centimeters long. Vessels in the bole frequently were many meters long. The maximum length of vessels found in the stem increased or diminished as the injection point varied between these limits. The extent of vascular invasion, which developed by the end of the season from June infections at various points in standing trees, was markedly conditioned by vessel length at the point of treatment. Spores injected 1 to 4 inches from the apex of new shoots produced no disease symptoms; after 2 years, invasion therein averaged 8.7 inches. Infections in the center of 1-year leaders or in crotches formed at the apex thereof induced extensive vascular invasion in 6 per cent of 323 treatments; in 20 per cent of 347 comparable treatments in 2-year stems. All treatments in the bole induced extensive vascular invasion. Invasion was limited to several feet in trees 60 feet high, girdled every 5 feet, injected at stump height in June and left standing all season.

A New Leaf Spot and Canker of Gardenia. BARRETT, J. T. In June, 1940, an apparently undescribed leaf spot and canker of Gardenia was observed in a single glass house near Oakland, California. It now (November 1) occurs in several houses but has not been observed in other locations. The leaf spot was first thought to be a severe occurrence of the leaf spot sometimes caused by *Phomopsis gardeniae.* Close examination, however, revealed on the underside of well-developed spots fungus fruiting bodies distinct from *Phomopsis.* Associated with the leaf spot were numerous cankers, mainly at the nodes of young shoots and most abundant on those near to or reclining on the ground. These cankers frequently originate from infected bud sheaths that persist at the stem nodes. Leaf spots vary in color from light to dark brown to dirty green, and, in size, up to 2 cm. in diameter. The spore masses, held together by mucous and dark green, surrounded by cottony white hyphae, are very conspicuous. Sometimes fruiting bodies occur on cankers. Repeated isolations of the organism have been made and its pathogenicity established. Leaf infection appears to take place in the absence of wounds. At the present time the pathogen seems to be an undetermined member of the Tuberculariaceae.

Experiments to Control Damping-off in Tomato Seedlings Transplanted from Sand. BEACH, W. S., AND SHUK YEE CHAN. Tomato seedlings started in sand washed free of humus by hot water are susceptible to damping-off when transplanted to nontreated soil. Varying the kind or the proportion of nutrient salts added to the sand does not affect susceptibility if the seedlings are transplanted in the seed-leaf stage, according to usual practice. Adding excess potassium sulphate, including minor elements, or using low water supply failed to increase resistance significantly. In experiments with soil inoculated with *Pythium* or *Rhizoctonia* from tomato, results show that effective amounts of copper sulphate and Semesan (30 per cent hydroxymercurichlorophenol) can be applied as drenches to prepared flats a day before or just after transplanting without noteworthy injury to the plants. In tests of copper sulphate ranging from 0.1 to 0.9 g. per sq. ft. of soil surface, significant growth stimulation usually occurred, but disease control was better with the higher applications and the more effective against *Pythium.* Semesan, 0.4 to 0.7 g. per sq. ft., gave good control of both fungi. In these experiments, the likelihood of growth retardation appeared to be overcome by plentiful watering for a few days after transplanting.

Shoot Enations on the Midrib and Petiole of Nicotiana glutinosa. BEALE, HELEN PURDY. Enations on the upper surface of the leaves of *Nicotiana glutinosa* have appeared sporadically in the greenhouses for the past 4 years. These excrescences vary from leafy frills complete leaf with petiole to entire shoots arising from the midrib, petiole, or veins. Often a portion of a vein becomes enlarged and may give rise to smaller, irregularly shaped enations. Conclusive evidence as to the cause of these abnormalities is still lacking. However, 4 separate outbreaks of these abnormal enations have been associated with the presence of mites. Small flies constantly present on the leaves are under investigation and the possibility of a virus being the causal agent has not been overlooked.

Hereditary Variation in the Ability of the Clover Leaf Hopper to Transmit Potato Yellow-dwarf Virus. BLACK, L. M. By selective breeding through 10 generations, 2 races of clover leaf hoppers (*Aceratagallia sanguinolenta*) have been obtained, one "active," the other "inactive." Under conditions where 80 per cent of the active individuals transmitted the potato yellow-dwarf virus (*Marmor vastans*) to crimson clover (*Trifolium incarnatum*), only 2 per cent of the inactive insects and 30 per cent of the hybrids between the two races transmitted. Infective individuals appeared in colonies of the inactive race, although all their ancestors for 10 generations had failed to transmit. Conversely, in colonies of the active race some insects failed to transmit, despite the fact that all their ancestors for 10 generations were infective. Infective insects in the active race were more efficient vectors (efficiency coefficient = 0.68) than infective insects in the inactive race (efficiency coefficient = 0.53). Male infective insects were slightly but significantly more efficient vectors (efficiency coefficient = 0.68) than female infective insects (efficiency coefficient = 0.64). A slightly but significantly higher percentage of males (43 per cent) than females (38 per cent) transmitted the virus. Tests with the hybrids indicated that sex-linkage may be involved in the heredity of ability to transmit.

Partial Purification of Potato Yellow-dwarf Virus. BLACK, L. M. The longevity of potato yellow-dwarf virus (*Marmor vastans*) in juice of *Nicotiana rustica* was greatly enhanced by cooling to 0° C. Freezing diseased leaves destroyed some virus. In purification experiments, diseased leaves were cooled to 0° C., and in subsequent procedures the virus was held as close to this temperature as possible without freezing. The leaves were ground, Na_2HPO_4 added to neutralize the pulp, and the juice expressed through cheesecloth. Partial purification was obtained by alternately filtering out coarse material on celite and sedimenting the virus in an ultracentrifuge. The virus was resuspended in neutral 0.1 M phosphate buffer. Yellow-dwarf virus sedimented much more rapidly in the ultracentrifuge than does tobacco-mosaic virus (*M. tabaci*), indicating that the infective agent is relatively large. Partially purified virus was more stable than nonpurified virus. Active virus could be demonstrated in partially purified preparations held 1 month in buffer at 0° C. Virus held at 0° C. was inactivated within an hour at pH 4 and pH 12, but more slowly at intermediate hydrogen-ion concentrations, the virus being more stable on the alkaline than on the acid side. Partly purified virus was passed through a Berkefeld W filter.

Relation of Soil Temperature to Armillaria Root Rot in California. BLISS, DONALD E. Plants were grown in a greenhouse in 5 Wisconsin tanks, with constant soil temperatures from 10° to 38° C. A pure culture of *Armillaria mellea* on root segments of *Citrus* was placed in the soil after the plants were well-established. After 14 months, seedlings of *Citrus sinensis* and *C. aurantium* (sweet and sour orange) showed severe infection at 10°, moderate infection at 17°, and slight infection at 24°. After 7 months, seedlings of *Prunus persica* (Lovell peach) and *P. armeniaca* (Royal apricot) and rooted cuttings of *Pelargonium hortatum* (geranium) showed moderate infection at 10° and severe infection at 17° and 24°. At 31°, the inoculum was not viable and all roots were healthy; at 38°, the roots died also. Greatest root growth of *Citrus* occurred at 24° and 31°; that of peach at 10° and 17°; and that of apricot and *Pelargonium* at 17°. Rhizomorphs of *Armillaria* developed in glucose-potato agar at 10° to 31° with optimum temperatures between 20° and 24°. Mean weekly soil temperatures in a citrus orchard at Anaheim, California, in 1939, ranged from 9.4° to 29.4° at 6 inches and from 13.3° to 20° at 8 feet.

A Method of Determining Loss in Yield Caused by Diseased or Missing Plants and Trials with Leaf Roll of Potato. BLODGETT, F. M., PHARES DECKER, AND C. S. TUTHILL. The plants in a row may be divided into 6 classes, depending on whether they are healthy (H) or diseased (D), and on the condition of the adjacent plants. Classes may be made as follows, it being understood that only the central plant of the group is being classified: HHH, DHH, or HHD, DHD, DDD, DDH or HDD, and HDH. The frequency of each of these classes for any percentage of disease would be, respectively, in order q^3, $2\ pq^2$, p^2q, p^3, $2\ p^2q$ and pq^2, in which p equals the fractional part of the plants diseased and q equals 1–p. Field results involving the leaf roll of potatoes proved that adjacent healthy plants do make up in part for the losses attributable to diseased plants, but that diseased plants are influenced less, or, not at all, by the condition of adjacent plants. On peat soil for Chippewa potatoes the yields in pounds per hill for the classes as above in order were 2.06, 2.20, 2.41, 1.22, 1.26, and 1.22; for Cobblers similar figures are incorporated in an equation to give the estimated yield (Y) of similar potatoes with any percentage of disease. $Y = 1.54\ q^3 + 1.70(2)\ pq^2 + 1.89\ p^2q + .72\ p^3 + .78(2)\ p^2q + .65\ pq^2$

Peach Mosaic in Western Colorado. BODINE, E. W., AND L. W. DURRELL. Strains of the peach mosaic virus have been observed in orchards at Palisade, Colorado. On the basis of symptoms, these strains are designated as severe, medium, and slight. There is possible also a fourth strain, referred to as slight, whose infectious nature has not been demonstrated. Working cooperatively, the Colorado Agricultural Experiment Station, the State Department of Agriculture, and the U. S. Bureau of Entomology and Plant Quarantine removed diseased trees in early spring and throughout the growing season for the 6-year period, 1935–1940, inclusive. Information was obtained regarding prevalence of these strains. Observations indicated that peach mosaic induced by the severe strain of the virus had been reduced from 95 per cent of the total number of diseased trees in 1935 to 10 per cent in 1940. Plants affected by the medium strain of the virus increased from 5 per cent in 1935 to approximately 50 per cent in 1938 and decreased to 40 per cent in 1940. The incidence of peach mosaic caused by the slight strain of the virus, apparent as a trace in 1936, remained constant (4 to 5 per cent) from 1937 to 1940. The number of trees showing symptoms caused by the strain designated as slight, which first appeared in 1938, increased to approximately 45 per cent of the affected trees in 1940.

Variations in Fusarium lini. BORLAUG, N. E., AND J. J. CHRISTENSEN. Isolates of *Fusarium lini*, obtained from many regions in the United States and Canada, differ greatly culturally and parasitically. Studies indicate that *F. lini* comprises numerous races some of which differ widely in pathogenicity on flax varieties previously or now grown in the Northwest. Bison is resistant to all races, except 6W, while Linota and North Dakota R114 are susceptible to most races. Pergamino No. 6098, an Argentine variety, is resistant to all races of *F. lini* tested, but very susceptible to an unidentified species of Fusarium, to which Bison also is susceptible. Chippewa and Winona, distributed previously as resistant varieties, but now susceptible in the field, have retained their resistance to some of the races of *F. lini*, but are highly susceptible to others. Some races are relatively stable in culture; others give rise to numerous variants that differ from the parental types in cultural characters and parasitism. The results indicate the importance of testing new varieties of flax to as many races of the pathogens as possible before they are generally distributed, since the breakdown of resistance may be attributed to the introduction or development of new physiologic races of *F. lini*.

Wind Dissemination of Angular Leaf Spot of Cotton. BROWN, J. G. Wind dissemination of angular leaf spot (*Phytomonas malvacera*) was demonstrated in an area of 1100 acres of cotton. The infective material was carried in dust. Cotton plants, wounded by a hailstorm 7 days before the windstorm, became extensively infected. Attendant circumstances contributed to make the field study fairly comparable to a planned laboratory experiment.

The Flexuous Hyphae of Puccinia graminis and Other Uredinales. BULLER, A. H. REGINALD. Flexuous hyphae were discovered in 1933 by Craigie. He saw them in the pycnia of *Puccinia helianthi* and *P. graminis;* and, in *P. helianthi*, he observed that, after the nectar of (+) and (−) pustules has been mixed, the flexuous hyphae fuse with pycniospores presumably of opposite sex. The writer has confirmed Craigie's findings and has observed fusions between pycniospores and flexuous hyphae not only in *Puccinia helianthi*, but also in *P. graminis, P. coronata,* and *Gymnosporangium clavipes.* The number of fusions observed in *P. graminis* was between 80 and 100. Flexuous hyphae are constant constituents of the mature pycnia of the Uredinales in general. The author has seen them in 30 species included in 8 genera. In *Puccinia, Uromyces,* and *Gymnosporangium* they are accompanied by numerous, straight or slightly bent, slenderly conical, pointed periphyses, whereas in *Phragmidium, Gymnoconia, Cronartium, Melampsora,* and *Melampsorella* they are not accompanied by periphyses.

Urediospores as the Origin of Systemic Mycelia in Puccinia suaveolens. BULLER, A. H. R., AND A. M. BROWN. *Puccinia suaveolens* is an autoecious brachy-form that parasitizes *Cirsium arvense* in Europe, North America, and other parts of the world. The mycelium perennates in the root-system. In the spring, in the field, the mycelium grows up into the new shoots and, on the leaves, froms numerous pycnia followed by uredia and some teliospores. The authors sowed urediospores on thistle seedlings, and soon secondary uredia were formed on the cotyledons and first foliage leaves. After several months, adventitious buds on secondary horizontal roots of the inoculated plants grew up into new green shoots, and the leaves of these shoots bore pycnia followed by uredia. Sometimes, however, the infected new shoots bore no pycnia and gave rise to uredia only. In one instance a new shoot bore pycnia only. When nectar from other infected plants was mixed with the nectar of the shoot that bore pycnia only, urediospores were formed at the points of mixing. This indicates that *P. suaveolens* is heterothallic.

A Fungous Parasite of the Mealy Bug. Charles, V. K., J. N. Couch, J. G. Harrar, and J. J. McKelvey, Jr. In August, 1939, mealy bugs (*Pseudococcus comstocki*) that appeared to have been killed by some fungus were found in Roanoke, Virginia. At that time, isolation experiments were unsuccessful, and similar material was not found again until August, 1940, when nearly all the adult mealy bugs in orchards near Roanoke were killed. At the same time, infected mealy bugs also were collected at Winchester, Virginia. From diseased mealy bugs a fungus, pathogenic for the species, has been isolated. Pure-cultures of the pathogen have led to the conclusion that it is an undescribed fungus. Through infection experiments mealy bugs have been readily killed in the laboratory and greenhouse. Subsequently, the pathogen has been reisolated from insects thus killed and used in further infection experiments. The mode of entrance, action, and transmission of the parasite have been studied and, at present, its potentialities as a biological control of the mealy bug are being investigated.

The Incidence of Purple-top Wilt in Pennsylvania on Certain Potato Varieties. Burke, O. D. The disease called purple-top wilt, or blue-stem, in Pennsylvania, has for many years caused serious losses both in yield and quality. It can be readily recognized on the tubers by characteristic necrosis at the stem end. Data on the incidence of tuber expression of purple-top wilt were taken in variety tests in 1939 and 1940. Differences were sufficiently great to place varieties in groups according to apparent susceptibility. Pennigan, Sequoia, Green Mountain, and Russet Rural were very susceptible. Chippewa, Katahdin, and Houma came in the medium group, while Cobbler, Mesaba, Earlaine No. 2, and Pontiac were in the least-susceptible group. Sebago had fewer tuber symptoms than any of the above varieties.

Custom Operation of the Oklahoma Hot-water Grain Seed Treater. Chester, K. Starr. A portable machine for hot-water seed treatment to control loose smuts of wheat and barley was designed and a working model tested in 1939 (Okla. Exp. Sta. Cir. 86. 1940). In 1940 a full-size machine, capable of treating 500 bushels of grain per day, was constructed for $250 and operated in 14 communities, treating 1927 bushels of grain for 130 farmers and 2 experiment stations. The machine combines advantages of semiautomatic heat control, automatic treatment-time control, rapid and thorough heat penetration, and portability. It is powered by steam and electricity effectively supplied by cotton gins, mills, power plants, creameries, and steam tractors. Instructed by county agricultural agents or vocational agriculture instructors, the growers presoaked their grain in seed-block quantities before treatment. It was dried either after return to the farm or on sections of paved roadway. Service charges of 5 or 10 cents per bushel paid the costs of operation ($130). Germination of 33 random grain samples after treatment averaged 93.5 per cent, with 19 samples showing 95–98 per cent healthy germination. The growers served were uniformly interested, cooperative, and satisfied. No essential modification of the machine is required in the extension of its operation planned for 1941.

The Identity and Relationships of Wheat Leaf-rust Races Collected in Oklahoma in 1939. Chester, K. Starr, and Lawrence Temple. Identification of 100 leaf-rust collections in 1939 yielded, in order of decreasing prevalence, races 13, 77, 2, 31, 58, 105, 20, 28, 52, 5, 9, 15, and 84. Since 1938, this indicates a marked relative increase of races that can attack Mediterranean and Democrat wheats, and that cannot injure Carina and Webster. Material increases were seen in race 77 (attacking all differential varieties), and race 105 (attacking all but Carina and Webster). The increase of races attacking Mediterranean is important, as this has been a commercial variety with considerable leaf-rust resistance. The group of environmentally variant races 13 = 9 = 19 previously proposed should also include races 20 and 31. Side-by-side culture of these races has shown that at one time both 13 and 31 react as 31; on another day, both as 13; while at other times races 20 and 9 react as race 31. To avoid future confusion from environmentally conditioned differences in identification, it is proposed that standard cultures of each race be maintained and distributed from one point, and that all described races be considered provisional until verified by comparison with such standard cultures.

Loss of Sporulation in Colletotrichum destructivum. Chilton, S. J. P. Single-spore cultures of *Colletotrichum destructivum* lost most of their ability to sporulate when kept in culture. Studies were made by continual single sporing of 14 cultures and the original sporulating types were retained in culture more than 2 years. Poorly sporulating variants from each were secured. As many as 15 variant types arose from an original single spore culture, some of which sporulated more profusely than the original types. Old tube cultures produced more variant types than fresh ones when single-spored. Staining of spores showed them to be predominantly uninucleate, with a few binucleate. Poorly sporulating variants did not regain their ability to sporulate after 3 passages through red-clover seedlings. Two variants were secured from a sporulating culture grown on red-clover seedlings under aseptic conditions, one of which produced very few spores. It is concluded that the loss of sporulation is attributable to the occurrence of poorly sporulating variants in the cultures, the variants probably arising through mutation.

Soil Treatments for Tobacco Plant Beds. CLAYTON, E. E., J. G. GAINES, G. M. STONE, AND K. J. SHAW. The control of weeds and that of the soil-borne diseases black root rot (*Thielaviopsis basicola*) and root-knot (*Heterodera marioni*) are major plant-bed problems for tobacco growers. Steam sterilization has been the only method effective against all three. Experiments conducted in 1939 at 5 locations and with a number of chemicals showed best results with cyanamid and chloropicrin. As a herbicide, cyanamid gave significant reductions in 10 out of 12 tests, chloropicrin in only 9 out of 18 tests. As a fungicide for the two diseases, cyanamid was ineffective and chloropicrin highly effective. A combination of 1 lb. cyanamid and 36 cc. of chloropicrin per square yard gave significant reductions in weed populations in 6 out of 6 tests and effective disease control in all tests. Compared with steam (pan) sterilization, the combination chemical treatment was slightly inferior in weed control and somewhat superior in disease control. In one experiment, plants produced with the chemical treatment were significantly larger and more drought-resistant.

Defoliation of Holly Cuttings by Rhizoctonia. COOLEY, J. S. A disease causing loss of holly cuttings in sand at the greenhouse, U. S. Horticultural Station, Beltsville, Md., has been found attributable to *Rhizoctonia solani.* The pathogen was isolated and the pathogenicity determined by the usual methods. The first symptom of the disease is the adherence of grains of sand to the under side of those leaves touching the sand, the fungus filaments with the adhering sand giving the effect of cobwebs. Two or 3 weeks after setting the cuttings, defoliation begins and advances very rapidly till 50 per cent or more of the cuttings may be killed. Treatment of the cutting bed, prior to setting the cuttings, with such substances as Bordeaux mixture, aluminum sulphate, cuprous oxide, ferrous sulphate, acetic acid, hydrochloric acid, sulphuric acid and crude phosphoric acid gave some measure of control; but the margin of safety was too narrow to justify recommending any of the chemicals tried. Satisfactory control was effected by setting the cuttings in fresh or sterilized sand and using precautions to maintain sanitary conditions. By this method a disease-free bed was maintained adjacent to a bed that was heavily inoculated with the pathogen.

Recent Developments in Methods of Distinguishing Wood Rots. DAVIDSON, ROSS W., W. A. CAMPBELL, AND ELMER R. ROTH. Methods of isolating and distinguishing wood-decaying fungi in culture were developed during a study of heart rots of oaks (*Quercus* spp.) and of 48 fungi associated with them. These methods have been applied also in a recent study of heart rots of merchantable black cherry (*Prunus serotina*). A printed index card has been devised for recording the growth characteristics of the fungi cultured. These cards can be filed according to key patterns based on these characteristics and compared with others on which have been recorded the characteristics of cultures from identified sporophores. Many species of fungi from indistinguishable heart rots have readily distinguishing characteristics in pure culture.

Anthracnose of Camellia. DAVIS, B. H., AND P. P. PIRONE. A canker of *Camellia japonica,* known to occur in New Jersey, Ohio, and Pennsylvania, is characterized by wilting and blighting of the foliage and by slightly sunken and sometimes blackened stem cankers, which are seldom noticed until wilting occurs. The inner tissues of the bark and the wood become dark brown. The pathogenicity of a fungus isolated consistently from such cankers has been established. The pathogen is considered to be specifically identical with *Glomerella cingulata,* reported by Shear and Wood on Camellia leaves. Its conidial stage is probably the same as the *Gloeosporium,* reported by Thelma B. Post as pathogenic and as the probable primary factor in death of Camellia twigs. Cross inoculations with isolates from Camellia and apple on potted young Stayman apple trees gave negative results with the Camellia isolate. Negative results were obtained with the apple fungus on Camellia stems, whereas positive results occurred with both isolates on apple fruits and camellia leaves. Tissues were wounded in all inoculations. The disease, although destructive during long periods of high humidity, has been checked by cutting out diseased stems and by surgical treatment of the cankers on large branches.

Mechanism of Invasion of Tobacco Leaves by Bacterium tabacum. DIACHUN, STEPHEN, W. D. VALLEAU, AND E. M. JOHNSON. A bacterial suspension of *Bacterium tabacum* placed on the surface of leaves of greenhouse or field-grown tobacco failed to produce wildfire, even if the leaves were kept moist several hours after inoculation. But infection did occur if the leaf tissues were flooded or water soaked, naturally or artificially, at the time of inoculation. Sustained water soaking is not necessary. If the water-soaked tissue was allowed to lose the excess water before inoculum was applied infection did not result. Furthermore, if the leaves were forcibly sprayed with sterile water, but not visibly water-soaked, just prior to inoculation, some bacteria invaded the tissues to produce typical wildfire spots. Infection also resulted when leaves were forcefully sprayed with a bacterial suspension. The authors interpret these results to invasion of tobacco leaves by *Bact. tabacum* can occur only if the stomata are open, and a water passage (visible as flooded or water-soaked tissue, or invisible) exists between the outer leaf surface and the intercellular spaces, or if the bacteria are injected forcefully through open stomata.

Measuring Inoculum Potential and Coverage Index of Sprays. DIMOND, ALBERT E. Inoculum potential measures disease-producing power of parasite and environment. Coverage index measures the completeness with which the leaf surface is covered by a deposit of fungicide. When percentage of disease, weighted by probits, is plotted against the logarithm of the fungicide dosage, a straight line results. The slope of this line is determined by 3 variables: fungicide, inoculum potential, and coverage index. With constant inoculum potential and coverage index, the slope is characteristic of the fungicide. With standardized coverage index and fungicide, slope measures inoculum potential. For apple scab, as inoculum potential advanced with the season, slope also increased. For tomatoes at one inoculum potential, slope was determined by plotting disease incidence, weighted by probits, against the logarithm of the toxicant dosage per acre for 3 gallonages. That the resulting 3 slopes, measuring coverage index, may be plotted against gallonage applied to give a straight line, is indicative of the rate at which increased protective action results from higher effective coverage of the plant by fungicide. These relations are useful (1) in following development of disease, (2) in field evaluation of fungicides, and (3) in predicting the amount of toxicant that should be applied to check advance of disease at any time.

Bactericidal Efficiency of Sodium Hypochlorite and Other Chemicals against Phytomonas sepedonica. DORRELL, WILLIAM W., E. A. MARTEN, AND J. G. LEACH. The bactericidal effect of phenol, copper sulphate, bichloride of mercury, sodium hypochlorite, and formaldehyde was tested on 15 cultures of *Phytomonas sepedonica.* Of these, sodium hypochlorite and bichloride of mercury were the most effective. The comparative value of these and other chemicals in seed treatment could not be determined because, in numerous greenhouse and field experiments, using several methods of inoculation, the amount of successful infection was extremely small. The results raise some question concerning the methods by which seed stocks become infected. Extensive morphological, cultural, and physiological studies showed that all of the cultures used corresponded with the descriptions of the pathogen found in the literature.

Factors Influencing the Movement of Viruses in the Potato. DYKSTRA, T. P. Studies on the movement of Canada streak and rugose mosaic in the potato have shown that these viruses can move through the parenchyma cells, but the major movement appears to be through the phloem, and the rate of movement is influenced by conditions affecting the flow of elaborated food particles. Removing the external and internal phloem of potato and tobacco plants failed to keep the virus from passing through the ring. Keeping the inoculated shoots in darkness caused a retardation of movement into the noninoculated part of the plant from 2 to 4 weeks and varied with individual plants. Virus can move from one shoot into another through the vascular tissue of the seed piece but not through the parenchyma. Virus can be recovered from inoculated leaflets after 96 hours but not from the noninoculated ones of the same plant before these develop symptoms. It requires 72 hours for the virus to move from the inoculated leaves into the petiole and 96 hours to move into the stem. The virus can be recovered from the stem 12 days after inoculation but not before that, even if stem parts are incubated for 2 weeks after removal from the plant.

Bacterial Wilt of Dent Corn. ELLIOTT, CHARLOTTE. Losses caused by bacterial leaf blight in new dent-corn hybrids in the Middle West in the past 2 seasons, have indicated the importance of tests of dent-corn inbreds for susceptibility to bacterial wilt. In the past two seasons at Arlington Farm, Virginia, between 800 and 900 such inbred lines have been inoculated, when 10 to 12 inches high, to determine differences in susceptibility or resistance to the disease in early stages of growth. The results have been compared with leaf blight ratings taken as the plants were nearing maturity. These records of the disease in early and late stages of growth have indicated that plants, resistant in early stages, may be susceptible to leaf blight as they approach maturity, and that most dent-corn inbreds are more susceptible to leaf blight in late than in early stages of growth. Inoculations of young plants apparently cannot be depended upon to indicate resistance to leaf blight, which is the important phase of this disease on dent corn in the field.

A Comparison of the Transmission of Four Cucurbit Viruses by Cucumber Beetles and by Aphids. FREITAG, J. H. Four cucurbit viruses occurring naturally in California were studied to determine their most efficient insect vectors. The squash-mosaic virus was readily transmitted by the western striped cucumber beetle, *Diabrotica trivittata* and the western twelve-spotted cucumber beetle, *Diabrotica soror* but infrequently by aphids. The striped cucumber beetle infected 23 of 25 squash plants, the twelve-spotted cucumber beetle 8 of 25, and of 8 species of aphids tested, only 5 species transmitted the virus and together infected only 13 of 245 plants. The cucurbit-ring-mosaic virus was transmitted by the striped cucumber beetle, which infected 18 of 56 squash plants, and the twelve-spotted cucumber beetle, which infected 4 of 21 plants, while 7 species of aphids failed to infect 250 squash plants. The wild cucumber (*Echinocystis fabacea*) mosaic virus was transmitted by the striped cucumber beetle, which infected 5 of 32 squash plants, while 9 species of aphids failed to infect 255 squash plants. Western-cucumber-mosaic virus was transmitted to only 1 of 61 squash plants by the striped cucumber beetle, while the twelve-spotted cucumber beetle failed to infect 30 squash plants. Additional tests, however, will be necessary with both species of beetles. Ten species of aphids readily transmitted the western-cucumber-mosaic virus to 104 of 271 squash plants.

Insect Transmission, Host Range and Properties of Squash-Mosaic Virus. FREITAG, J. H. The western striped cucumber beetle, *Diabrotica trivittata,* was a more efficient vector of the squash-mosaic virus than the western twelve-spotted cucumber beetle, *Diabrotica soror.* When 10 beetles were transferred to each squash plant for a period of 1 day, the striped cucumber beetle infected 23 of 25 plants and the twelve-spotted cucumber beetle 8 of 25 plants. Single striped cucumber beetles infected 19 of 30 squash plants and single twelve-spotted beetles 2 of 25 plants. Striped cucumber beetles, first fed on diseased plants for several days, then transferred daily to successive healthy squash plants, remained infective for a period of 10 days. The virus was recovered from the crushed bodies of the striped and twelve-spotted cucumber beetle that had been feeding on disased leaves and also from the crushed bodies of infective striped cucumber beetles following a 5-day feeding period on healthy squash plants. A study of the physical properties of the virus shows that the virus was infectious when diluted 1: 1,000,000, was still active after 6 weeks *in vitro* at room temperature or 6 months at 0° F., and was inactivated at 75° C. in 10-minute exposures. The host range determined up to the present is limited, and the virus has been transmitted and recovered from 15 species of plants in 11 genera belonging to 4 families.

Strain Combinations of Curly-top Virus. GIDDINGS, N. J. Seven clearly differentiated strains of curly-top virus were used. Sugar beet plants infected for at least a month with one virus strain were inoculated with a second strain. Subsequent tests determined the presence or absence of the second strain, and whether any changes in virulence had occurred. Seven strains tested in the above manner permit 42 possible combinations. Fifteen of these have been tested, and the second inoculation was positive in every case. Less virulent strains infected plants previously infected with the more virulent strains, and the more virulent strains were readily introduced into plants already infected by those less virulent. Strain combinations of approximately similar virulence likewise gave positive results. There have been no indications of changes in virulence. A sugar-beet plant infected with 2 virus strains of different virulence may carry the more virulent strain, but show only symptoms of the less virulent strain. At least 3 strains of virus may be carried simultaneously by 1 leaf hopper, and daily transfers of such an insect may give, consecutively 3 curly-top plants, each expressing typical symptoms of a different virus strain.

Compatibility of Various "Inert Ingredients" with Cuprous Oxide and Calcium Arsenate. GOODWIN, M. W., K. J. KADOW AND D. F. MURPHY. Eighty-one so-called "inert ingredients" were mixed individually with cuprous oxide (Cuprocide G. A.) and calcium arsenate at the ratio of 6 lb. cuprous oxide, 10 lb. calcium arsenate and 84 lb. "inert." Each dust mixture was allowed to stand for 30 minutes in an equal weight of distilled water, then pH values were taken and the mixture filtered. The filtrate was thereafter analyzed for water-soluble copper and arsenic. The pH values of the mixtures varied from 6.4 to 9.8. Water-soluble copper varied from none to 0.735 per cent, with most mixtures giving less than 0.1 per cent. Water-soluble arsenic, as As_2O_3, varied from 0.29 per cent (hydrated lime) to 8.44 per cent. There seems to be a definite correlation between the solubility of calcium arsenate and the pH value of the dust mixture, but no correlation is apparent between the solubility of the cuprous oxide and pH. The pH values of the inert ingredients alone gave no indication of their effect on the pH of the resulting mixtures due to differences in their individual buffering characteristics. No information is yet available regarding the relation of soluble copper and arsenic to plant injury. We do not know how much of these soluble materials different plants will stand without injury. These studies will be continued with other copper compounds.

Smut Resistance in Vegetable Species of Allium. Felix, E. L. Natural smut immunity of seedlings in the postcotyledonous stage and of mature bulbs of the common onion (*Allium cepa* L.) and bulbs of the yellow and white multiplier (*A. cepa* var. *solaninum*) is due to physical protection of the younger tissues by the surrounding older. The common and multiplier onions were infected readily by exposure of the plantlets within by cutting away the upper two-thirds of the bulbs, or better, by removal of the outer scales. On artificial exposure, plantlets from bulblets of the top onion (*A. cepa* var. *viviparum*) proved highly resistant, while those from cloves of garlic (*A. sativum* L.) were immune, indicating also inherent resistance. Cotyledons of the onion (*A. fistulosum*), leek (*A. porrum*) and chive (*A. schoenoprasum*) possess high and equal natural resistance to smut. The resistant factor (or factors) in leek and chive is distributed uniformly in the seedling (except in the root), as is the factor for susceptibility in *A. cepa*, while, in *A. fistulosum*, resistance of the cotyledon is far greater in the sheath than in the blade. Red Welsh (French Welsh) was highly susceptible and belonged to *A. cepa*. Species and not varietal differences in smut resistance prevail.

Use of Hardwood Oils and Other Chemicals in the Control of Onion Smut. Felix, E. L. Three-year experiments on muck soil indicate that onion smut may be reduced 63 per cent, without crop injury, by the drip application at the time of sowing of 200 gal. per acre of 3 per cent No-D-K (Tennessee Eastman Corporation) or by 100 gal. per acre of 2 per cent neutral oil, employing 0.2 per cent sodium lauryl sulphate (Grasselli IN 181) as a dispersing agent. Inexpensive neutral oil proved to be the most active ingredient of No-D-K against onion smut, while oil no. 45, another constituent, was relatively nontoxic to the fungus. DuBay 1286-x (50 per cent manganese dimethyldithiocarbamate) plus methyl orange (2 to 1) at the rate of 1½ oz. to 1 lb. of onion seed reduced smut 50 per cent without crop injury, and was more effective than pure tetramethylthiouram disulphide plus methyl orange or copper stearate (4 to 1) at the rate of 2 oz. to 1 lb. of seed. Sodium dimethyldithiocarbamate-drip (2 per cent) at the rate of 100 gal. per acre also reduced the disease 50 per cent. Similar reduction in smut was obtained in seed and soil treatments with 5-hydroxy-1, 3-dimethylbenzene, which is comparatively inexpensive but requires further development in methods of treatment.

Respiration of Conidia of Sclerotinia fructicola. Frampton, Vernon L., and Paul M. Marsh. A study of the respiration of the conidia of the brown-rot fungus, *Sclerotinia fructicola*, with the aid of the Fenn micro-respirometer is in progress. This apparatus is capable of measuring the oxygen consumption of a relatively small amount of spores, quantities easily obtainable in ordinary Petri-dish culture. The following provisional statements are based on experiments preliminary to a study of the mechanism of copper toxicity. The respiration rate of the dry, ungerminated spore is very high. The rate of oxygen uptake increases considerably within a few minutes after wetting and can be still further increased by the addition of either glucose, maltose, or sucrose. The appearance and rapid growth of the germ tube is not accompanied by any measurable increase in oxygen consumption. In the presence of 5 p.p.m. Cu as $CuSO_4$ the respiration rate decreases slowly to a relatively stable residual rate that persists for several hours.

The Role of Insects in the Pit Scab of Potatoes. Granovsky, A. A., and A. G. Peterson. Pit scab resembles insect injury, borders of which are lined with scab (*Actinomyces scabies*) infection. Weekly examinations by digging of potato hills for insects and scab infection from planting date to harvesting, show only a few pit scab lesions infested with insects. Infection takes place primarily through lenticels without the aid of insect injury. The cracking of the infected lenticels is accelerated by the rapid growth of tubers in the presence of soil moisture. This successive cracking repeatedly ruptures the wound cork beneath the small initial scab lesions, permitting mycelial ramification into deeper layers of the tuber. The affected potato tissue in scab pustules absorbs and holds moisture, prevents formation of effective wound cork, and provides a suitable medium for secondary saprophytic organisms, especially bacteria. Several forms of soil microfauna invade scab pits previously made by secondary decay, especially mites, *Macrochelus* sp., *Rhizoglyphus hyacinthi*, and *Rh. phylloxerae*, certain *Collembola* sp., and occasionally fungus gnats, *Sciara pauciseta*, and often early stages of Annelid worms. These insects and mites are largely scavengers in their food habits feeding on decaying organic matter produced by the mycelial action of scab and further decomposition by the secondary bacterial invaders. Mites, apparently, act as effective vectors of saprophytic bacterial organisms.

Peach Canker Caused by Fusicoccum amygdali. HAENSELER, C. M., AND R. H. DAINES. A canker of peach, which apparently has not previously been reported in America, has been under observation in New Jersey for several years. Twigs with the same disease have been obtained also from Rhode Island. The cankers occur almost invariably at the nodes of young but not actively growing twigs. They are usually 1 to 3 cm. long and extend half way, rarely entirely, around the twig. They start in the late fall or early spring as inconspicuous, water-soaked, poorly defined areas, but soon become oval, slightly sunken, brown, well defined cankers. Older cankers become hard, dry, and gray, and usually bear prominent, erumpent pycnidia that exude whitish spore tendrils in moist weather. Cankers on older branches and at base of current year's twigs may prove to be a phase of the same disease. The twig cankers may cause appreciable direct injury, but seem to be of greatest importance as infection courts for *Valsa* spp., which cause a very destructive twig blight. A *Fusicoccum* sp., constantly associated with the nodal twig cankers, has been isolated many times, and typical cankers have resulted from pure culture inoculations. The fungus is morphologically very similar to *Fusicoccum amygdali.*

The Parasitism of Economic Insects by Fungi. HARRAR, J. G., J. J. MCKELVEY, AND J. W. SHOWALTER. For several years collections of insects have been made and examined for the presence of parasitic fungi. Several such fungi have been isolated from aphids, leaf hoppers, mealy bugs, bark beetles and flies. Most of these fungi belong to the genera *Entomophthora, Beauveria, Conidiobolus,* and *Hirsutella,* but several have not yet been definitely placed. All those here considered have been found to be insect parasites. The several pathogens have been compared as to pathogenicity, mode of entrance and action, dissemination and host range. They also have been compared in culture in attempt to establish criteria for the separation of entomogenous species on a physiological basis. In progress are experiments to evaluate the effectiveness of each of these fungi in the biological control of economic insects.

Florescence of Potatoes under Ultraviolet Light for Detecting Ring Rot. HARVEY, R. B. Seed-potato-cutting machines, using the G. E. BH4 lamp, are described for use by inspectors, and also a practical hopper-type cutter for 4 knives. A lot of 100 bushels of White Rose, containing 0.5 per cent ring-rot tubers, was cut, rejecting tubers that fluoresced greenish, followed by immersion in Cinnex 20 and 1 per cent potassium permanganate solution. No ring rot was found after either sterilizing treatment on sampling the yield.

Association of Bacterium phaseoli and the Virus of the Common Bean Mosaic: the Effect of Varying Amounts of Nitrogen. HEDGES, FLORENCE, AND HERBERT FISHER. In an earlier study of serial passages of bean juice containing *Bacterium phaseoli* and common bean-mosaic virus, it was found by the senior writer that the virus symptoms predominated and, furthermore, that the bacterium underwent dissociation in the course of this long-continued association. There is here reported a study of the effect of nutrient solution with low, medium, and high nitrogen content on young bean plants grown in washed quartz sand and inoculated with juice from the serial passages and with the two infective agents alone. A parallel series was carried on in composted soil. Inoculum from a serial passage showing little or no bacterial infection, produced 100 per cent striking *Bacterium phaseoli* infection in the medium N set and but traces in the high and low N and soil sets. Isolations from all of the N sets were pathogenic, but that from the medium N was by far the most virulent. One hundred per cent mosaic appeared in all 4 sets, but developed the most rapidly in medium N and composted soil. Later experiments with *Bacterium phaseoli* cultures and inoculum from serial passages showing both bacterial and virus symptoms gave best bacterial infection in medium N or composted soil. The virus behaved similarly. Under the conditions of these experiments, optimum conditions for plant growth were optimum for both infective agents.

Copper Fungicide Tests on Tomatoes. HENDERSON, R. G., AND S. A. WINGARD. Tomato spray tests at the Virginia Agricultural Experiment Station in the current season consisted of the spraying of Earliana, Pritchard, and Marglobe tomatoes with Bordeaux mixture, yellow copper oxide, and tribasic copper sulphate and the dusting of the same varieties with red copper oxide and tribasic copper sulphate. With the exception of Bordeaux, each of these materials gave a highly significant increase in yield of fruit over the nontreated check. The increase in yield with tribasic copper sulphate spray, however, hardly equalled that with the other materials, but the difference was insignificant. Bordeaux also gave an increase, but was statistically insignificant. On the later maturing varieties, Pritchard and Marglobe, Bordeaux resulted in decreased yield, the greater decrease being for Marglobe, which was slightly later in maturing its fruit. All of the other treatments gave an increase in yield on both Pritchard and Marglobe, except the yellow copper oxide spray, which, on Marglobe, gave no increase over the nonsprayed check. Too much reliance, however, should not be placed on the data from the Pritchard and Marglobe varieties because the experiment was abruptly terminated by an early killing frost while much of the fruit was immature.

Timing and Dosage of Tomato Sprays for Alternaria Control. HUEBERGER, JOHN W. A timing test was made, using yellow cuprous oxide, in which sprays were added or omitted progressively at 2-week intervals, June 21–August 23, 1940, or made at selected mid- and late-season dates. Disease readings were made Aug. 23 and Sept. 7. Data obtained showed: 1. Time that data are taken affects the rating, *e.g.*, 2-spray schedules for July 19–26 and Aug. 2–9 had indexes of 2.58 and 2.75 on Aug. 22; whereas Sept. 7 indexes were 4.45 and 3.40; 2. Spraying should commence about mid-July, just before stage when plant falls over and Alternaria begins to attack, and continue through August, *e.g.*, on Sept. 7, a 6-spray schedule, ending July 26, had index of 4.50; whereas a 4-spray schedule beginning as late as Aug. 2 had index of 2.70, the check index being 5.00 (maximum); 3. Spraying only during *Alternaria* development will reduce disease incidence, *e.g.*, index of 3.40 for a 2-spray schedule (Aug. 2–9). In a 4-spray-schedule experiment (July 24–Aug. 23) the optimum dosage per acre per application lay between 200 and 300 gallons and 3 and 4 pounds actual copper.

The Biology of Fomes fomentarius. HILBORN, M. T. Studies on the morphology of the sporophore show that the tube layers are stratified. The 1938 spore-discharge period was approximately 180 days. This discharge exhibited 3 phases, (1) the prepeak, (2) the peak, and (3) the postpeak. Atmospheric humidity was an influencing factor on spore discharge during the prepeak interval but not later. Temperature was an influencing factor on spore discharge during the peak interval. Cultural studies showed that there was considerable variation in the time-growth curve for various isolates. No subgroups could be classified from the isolates studied. The differences observed were apparently attributable to individual variation, which was independent of either host or locality. The fungus simultaneously causes typical heart rot and attacks the sapwood. Chemical studies on decayed wood showed that the fungus can attack lignin and cellulose simultaneously. All pentosans were utilized alike. A decrease in alkali solubility, characteristic of the white rots, was found. The losses in calorific value of the decayed wood were proportional to the losses in dry weight.

Mechanical Transmission from Currant to Tobacco of a Virus Identified as Annulus zonatus. HILDEBRAND, E. M. A virus recently transmitted to tobacco by mechanical means from plants with currant-mosaic symptoms has been identified as a highly virulent strain of tomato-ringspot virus, *Annulus zonatus.* This appears to be the first case of a virus in a woody perennial plant being transmitted to succulent annual plants. Extracts from currant leaves, showing either typical chlorosis or hitherto unobserved zonate chlorotic spots, when rubbed on young tobacco plants dusted wtih carborundum, have consistently produced zonate necrotic primary lesions, the number being larger on *Nicotiana rustica* than on Turkish tobacco (*N. tabacum*). Cross-immunization tests with other ringspot viruses on Turkish tobacco have resulted in protection against tomato ringspot only. Likewise, there is close agreement with tomato ringspot in other details, including host range, incubation period, thermal inactivation, longevity, filterability, and dilution endpoint. The virus was isolated from diseased currants in 14 out of 16 trials, whereas negative results were obtained in all 10 trials from healthy plants. Transmission of the virus from tobacco back to currant has not been successful. However, until this has been accomplished, it cannot be stated with certainty that the same virus is involved in current mosaic and tomato ringspot.

Micrurgical Studies on the Crown-gall Organism with Special Reference to the Infection Mechanism in Tomato. HILDEBRAND, E. M. Certain problems centering around infection of the tomato by the crown-gall organism have been attacked by micrurgical methods with interesting results. More than 90 per cent of single cells grew in sterile juice extract from young tomato plants. Single cells introduced into deep wounds on tomato stems produced galls in from 10 to 60 per cent of the trials as compared to 10 per cent or less with shallow wounds. Similarly when 10 or more cells were used gall formation resulted in about 90 per cent of the trials with deep wounds as compared to 20 per cent or less with shallow wounds. These results suggest that the kind of wound may be a more important factor than the number of bacteria. None of the more than 150 individual plant cells injected with numerous bacteria responded with galls. Living bacteria were recovered 3 weeks later from but one of 20 cells cultured. Wounds several times larger than the above, produced by gently rubbing a polished needle with bacteria on stems and petioles, resulted in tiny galls close to collapsed hairs. Very few bacteria were observed in these galls.

Quantitative Measurement of Tobacco-etch Virus. HOLMES, FRANCIS O. It has not been feasible heretofore to make extensive quantitative measurements of tobacco-etch virus (*Marmor erodens*); none of its recognized host plants have shown distinctive primary lesions. Recently, additional species were found susceptible. Inoculation tests were made of 220 kinds of herbaceous plants, in 50 families; 69 species in 11 families became infected. One species, *Physalis peruviana* was found to develop conspicuous brown, necrotic, primary lesions with darker peripheral pigmentation. By the use of this plant, etch virus can be identified readily, and quantitative studies can be pursued. It has been found that tobacco-etch virus in expressed juice is inactivated in 10 minutes at 55° to 57° C. The virus content of detached tobacco leaves decreases gradually during drying at room temperature until, about the time complete dryness is attained, no more virus is detectable. The virus increases rapidly in a recently infected tobacco plant, first in the inoculated leaf, then in the systemically infected top, reaching a maximum in both locations at about the tenth day; the concentration then decreases spontaneously, more rapidly in the top than in the inoculated leaves.

Buds as a Factor in the Overwintering of Shot-hole of Peach (Phytomonas pruni). HOPPERSTEAD, S. L., AND T. F. MANNS. Data obtained in 3 years from cultural and histological work in the laboratory and from field experiments and observations indicate that the terminal buds of the peach harbor the organism from one season to the next. There was no conclusive evidence that lateral buds were a factor. The percentage of terminal buds from which positive isolations could be obtained was quite high during the early part of the dormant season, but decreased rapidly as the season progressed. Cultures of *Phytomonas pruni* were obtained also from tissues showing no visible lesions along the last 10–12 inches of the twig near the tip end. In these tissues the percentage of positive isolations increased as the material was cultured progressively toward the tip. Pathogenicity tests were run on the cultures obtained. Only those showing positive results were considered *Phytomonas pruni.* Histological work showed the presence of bacterial masses in the intercellular spaces of the terminal buds.

Predicting Protective Value of Fungicides in the Laboratory. HORSFALL, J. G., J. W. HEUBERGER AND A. E. DIMOND. (1) Log-probit transformations of dosage-response data, from both field and laboratory, yield straight lines. (2) The slopes of these lines are characteristic of the fungicide. (3) Test-to-test variability in both laboratory and field data was reduced by using a fungicide and protective coefficient, respectively; ratios of the dosages of a standard and the test material at unit response. (4) Fungicidal value and tenacity, the two fundamental components of protective value, have been measured in the laboratory. (a) With both components constant, protective value was equal (Tennessee tribasic cooper sulphate = Basicop). (b) With tenacity equal, protective value followed fungicidal value (yellow > red cuprous oxide. Compound A > Cupro K). (c) With fungicidal value equal, protective value followed tenacity (Cuprous oxide > cuprous oxide plus goulac). (d) When both components vary, protective value can be predicted only by weighting the relative magnitudes. Tenacity probably will require the heavier weighting, because materials that do not stick well do not protect well, however fungicidal they are. (5) Field designs for evaluating fungicides improve in efficiency when made homologous to laboratory designs.

The Role of Tree Injection in the Control of Bleeding Canker of Hardwoods. HOWARD, FRANK L., AND NESTOR E. CAROSELLI. Discovery of beech, elm, and at least two species of oak (*Q. rubra* and *Q. agrifolia*) infected with bleeding canker, previously reported only on maple trees, has increased its importance. Confirmed diseased trees are present at various points in New England, on Long Island (N. Y.), and near Los Angeles (Calif.). A method of internal medication for checking the injury caused by this vascular pathogen will be demonstrated with Kodachrome motion pictures. "Helione orange," a proprietary basic yellow dye plus a penetrant, has been found *in vitro* to inhibit the growth of *Phytophthora cactorum* and other pathogenic fungi and to inactivate the toxin formed by them. In 1939 and 1940 over 349 confirmed diseased maple and 11 beech trees in the field were injected and 85.4 per cent of these have failed to yield the pathogen, have inactive cankers, and are forming callus tissue. Studies on the relationship of the point of injection, dosage, and the distribution of the chemical in the tree have shown that infected vascular and cambial tissues can be adequately reached. Evidence supports the theory that the chemical checks tree decline by inhibiting the fungus, and by neutralizing the mycotoxin, thus preventing wilting, decreasing browning of leaf margins and bud tips, and promoting greener leaves.

Ascochyta majalis Identified on Lily of the Valley in the United States. JENKINS, ANNA E. In August, 1940, a grower of lily of the valley in southeastern Pennsylvania became alarmed over the severely diseased condition observed in a plot of lily of the valley he had maintained in healthy condition for about fifteen years. Near the center of the plot, approximately 25 × 50 feet in size, an elongated area about 20 × 2 feet, was entirely discolored. A more or less general spotting of the green leaves outside this area was observable. On representative specimens submitted to the Department of Agriculture for diagnosis and referred to the writer for examination, there was found an imperfect fungus that proved identical with *Ascochyta majalis.* This fungus was discovered in October, 1899, by Massalongo on spots on nearly dead leaves of cultivated lily of the valley growing at Verona, Italy. In 1908, it was reported by Otto Jaap in the Austrian Alps. Only these two mycological records of the fungus are available, and, until found associated with the disease outbreak in the Pennsylvania planting its apparent importance as a disease-producing organism had not been recognized.

Transmission of Viruses by the Parasitic Activities of Dodder. JOHNSON, FOLKE. The union between a host plant and its dodder parasite resembles a graft union, the parasite being totally dependent upon its host for sustenance, just as the scion must depend upon its stock. A single dodder plant parasitizing 2 hosts forms a natural bridge of conducting tissue between them. Plants as far removed taxonomically as pteridophytes and spermatophytes may be thus connected. Last year, Bennett reported that cucumber-mosaic and sugar-beet curly-top viruses were transmitted by the dodder species, *Cuscuta subinclusa.* Recent tests by the writer have shown that the viruses of aster yellows, tomato bushy stunt, cucurbit mosaic, sugar-beet curly top, tobacco mosaic, and a mosaic disease of white clover may be transmitted from diseased to healthy plants by another species of dodder, *C. campestris.* The time required for symptoms to appear in the test plants after they have become parasitized by infected dodder is greater than that required for these viruses following mechanical or insect transmission. Tobacco-ringspot virus was not transmitted from diseased to healthy Turkish tobacco by this method. Neither tobacco-mosaic virus, which was transmitted, nor tobacco-ringspot virus, which was not transmitted, multiplies in dodder.

Ground Sprays Aid in Control of Apple Scab. KADOW, K. J., AND S. L. HOPPERSTEAD. In view of the recent interest in ground sprays, an effort was made this season to learn whether such applications were commercially feasible. To this end, experiments were conducted in Delaware on 5 different farms. The experiments involved a comparison of a 22.25 per cent sodium dinitro ortho cresylate (Elgetol) used at ½ per cent by volume; a 40 per cent sodium dinitro ortho cresylate (American Cyanamid Co.) at ¼ per cent by volume and sodium nitrate 100 lb. plus calcium arsenite 4 lb. with water to make 100 gal. In all cases these sprays were applied at from 500 to 600 gal. per acre. In 2 of the 5 orchards, ground sprays helped but little to control scab. Both of these failures are explained by improper supplementary spray programs and spraying technic. In the other cases good results were obtained. This season's work supports the following conclusions: 1. All 3 ground treatments were effective aids in scab control where ascospore inoculum was high. 2. Ground sprays were of no practical value where ascospore inoculum was low. 3. This season's ground-spray applications were about equal in their effect to 3 scab sprays. (They will vary greatly with the season and the variety under consideration.) 4. Ground sprays definitely cannot be relied upon to control scab without the aid of a spray program. 5. Ground sprays permitted the successful use of milder sulphur sprays than could otherwise have been successfully used.

Experiments with Eradicant Fungicides in Relation to Apple-scab Control. KEITT, G. W., C. N. CLAYTON, AND J. D. MOORE. The floor of an 8-acre block in an orchard in clover sod near Casco, Wisconsin, was sprayed at bud-break with Elgetol ½ per cent plus monocalcium arsenite ½ per cent, 300 gal. per acre. This block was well isolated from apple orchards receiving no eradicant spray. Disease development was followed on nonsprayed McIntosh trees in the treated block and in 2 nontreated orchards 0.3 and 0.5 miles away, respectively. The season favored severe occurrence of scab. Counts made on June 12, shortly after petal-fall, showed percentage reductions in lesions per leaf in the treated orchard as compared with the nontreated orchards of 99 and 95, respectively. The floor of a similarly isolated 4-acre McIntosh orchard in quack-grass sod was sprayed shortly prior to bud-break with Elgetol Extra 1 per cent, 400 gal. per acre. Counts made June 13 on nonsprayed McIntosh showed reductions in lesions per leaf in this treated orchard as compared with 2 small nontreated orchards of 89 and 97 per cent, respectively. Variations in the several orchards must be considered in interpreting these data. Comparative studies seeking to evaluate the ascosporic inoculums are in progress. Results of summer spraying will be reported elsewhere.

Further Experiments on a Virus Disease of Prunus cerasus. KEITT, G. W., AND C. N. CLAYTON. Budding experiments confirmed those reported last year (Phytopath. **30**: 13), the disease being readily transmitted from *Prunus cerasus* to *P. cerasus*. It is concluded that the inciting cause is a virus. The disease has been transmitted by budding from *P. cerasus* to *P. mahaleb* and back to *P. cerasus*. Buds from diseased *P. cerasus* were inserted in several sprouts of *P. virginiana*. Leaves of these budded sprouts developed conspicuous chlorosis the following year: the controls remained healthy. A similar experiment gave strong suggestion of transmission of the disease from *P. cerasus* to *P. pennsylvanica*. Records taken in 5 orchards (2593 trees) near Sturgeon Bay, Wisconsin, for 5 years showed an average annual increase of about 3 per cent in incidence of trees classed as moderately or severely diseased. These data, however, are not comprehensive enough to be taken as representative of the Door County district. Further studies are in progress, including work on variability in disease development and on possibilities for increasing the productiveness of diseased trees. It is recommended that measures be taken to avoid spread of the disease in nursery stock. Recommendations for combating the disease in the orchard are withheld until further experimental results are available.

Potato-ring-rot Studies in California. KENDRICK, JAMES B., P. A. ARK, AND C. EMLEN SCOTT. Field tests have shown the cutting knife, pickerplanter, and surface contact to be effective agents in transmitting ring rot. Healthy potatoes, cut with a contaminated knife, showed an average of 69.3 per cent diseased hills. Healthy seed lots, with 2 to 10 per cent diseased potatoes added before cutting, showed an average of 58.0 per cent diseased hills. Contaminated pickerspikes transmitted ring rot to 57.7 per cent of the hills. Diseased cut seed, mixed with healthy cut seed and whole potatoes planted after being dipped in a water suspension of macerated diseased potatoes, transmitted a high percentage of ring rot. Whole seed *versus* cut seed showed approximately 3 times more ring rot from cut-seed planting. Alternate diseased and healthy hill planting has not shown evidence of current-season spread. Soil carry-over of ring rot has not been demonstrated. Apparently healthy potatoes selected at harvest from diseased seed lots showed 7.3 per cent ring rot. Dipping contaminated cut seed in iodine, potassium permanganate, Semesan Bel, DuBay 1230-B.S., Cinnex 20, and Mercuronol solutions failed to control ring rot.

Effect of Cutting-bench Treatments on the Incidence of Disease in Carnations. KIRBY, R. S. Tests conducted in a commercial greenhouse in 1939 and 1940 showed that certain growth-promoting substances, applied as a dust to the base of Puritan carnation cuttings, reduced the incidence of Alternaria blight over the potassium permanganate soak by 29.4 per cent (28.6% *vs.* 58%). When the treatments were combined by first immersing the entire cutting for 10 minutes in 1–1000 potassium permanganate and then dipping the cut ends into the growth promoting substance (powder) the incidence of disease was further reduced to 8.3 per cent. Nontreated cuttings were 80.2 per cent diseased. The percentage of well-rooted cuttings and th average weight of cuttings increased as the percentage of diseased plants decreased. The 3 growth-promoting substances applied contained one of the following: (1) naphthalene acetic acid, (2) naphthyl acetamide, and (3) indolebutyric acid.

Cure of Aster Yellows by Heat Treatments. KUNKEL, L. O. Previous reports have recorded the cure of 4 virus diseases of peach and 2 of sugar cane by heat treatments. To this list may now be added aster yellows in the periwinkle, *Vinca rosea*. The plants have been cured by treatments in which temperatures of from 32° to 42° C. were employed. In some of the treatments potted plants were submerged in a water bath, while in others they were incubated in a hot room. Neither method of treatment caused serious injury. Both methods were effective. Growth produced subsequently to cure was normal in every respect. Cured plants were as susceptible to a second or a third attack of the disease as were plants that had never had yellows. Since no virus could be obtained from plants that had received adequate treatments, the cures were believed to have resulted from heat inactivation of yellows virus. Aster yellows probably can be cured in all species capable of enduring moderately severe heat treatments.

Bacterial Ring Rot in Relation to the Tomato. LARSON, R. H., J. C. WALKER, AND S. O. FOGELBERG. The tomato (*Lycopersicum esculentum* var. Bonny Best) is easily infected by needle inoculation with bacterial ring rot of potato (*Phytomonas sepedonica*). In the greenhouse the study of temperature relations shows that symptoms on inoculated plants develop most rapidly between 24° and 28° C. and are suppressed at 16° C. and below. Early symptoms at 24°–28° are evident in 12 to 14 days as a slight loss of plant color, upward and inward marginal leaflet rolling, and wilting. As the leaflets become permanently wilted, shrivelled, and moribund, irregular necrotic streaking of the stems occurs. This often is followed by a creamy, light-colored, slimy bacterial exudate at points of tissue breakdown. In vascular elements the bacteria progress primarily through the xylem vessels and thence progress intercellularly into the parenchyma of pith and cortex. Cross sections of diseased stems show pith cavities. In the systemically infected fruit, external symptoms appear near or at the pedicel as irregular soft water-soaked areas. Interval change is evident as tissue breakdown, in the small size and number of immature seeds, and in light-colored bacterial masses in the diseased area. The bacteria have been recovered in pure culture from infected seeds and histologically demonstrated. The planting of seeds from infected fruit has yielded infected seedlings. The possibility of the tomato as an important agent of transmitting the potato ring-rot organism is suggested.

Experimental Production of Invading Overgrowths in Plants. Levine, Michael. Animal cancer is characterized by extension of malignant cells into normal tissue of the host. Crown-gall tissue has sporadically shown evidence of invasion, although the malignant nature of the growth has not been considered analogous to animal neoplasia. An effort was made to induce plant overgrowths to assume an invasive character. More than 500 inoculations with a virulent strain of *Bacterium tumefaciens* and treatments after injury with indoleacetic, indolebutyric and α-naphthaleneacetic acids were made on 15 species of garden and house plants. The injured and treated areas were then tightly covered with parafilm and wound with adhesive tape. Treated areas of control plants were left uncovered. *B. tumefaciens* produces large crown galls on Ricinus, dahlia, broccoli, geranium, Kalanchoë, and chicory. In but few cases can the invasive crown-gall tissue be attributed to application of pressure over the inoculated area. Growth substances induce swelling and root formation. Under the parafilm and adhesive tape covering, Ricinus stems form many stubby, root-like structures, while the sunflower produces long, branching roots. Intumescences and roots form on the surface of the chicory stem, and the lumen, as observed in Ricinus, becomes filled with parenchymatous tissue. Severity and depth of injury in treatment is more effective in producing invading growths than is pressure.

Bacterial Necrosis of the Giant Cactus, Carnegiea gigantea (Cereus giganteus). Lightle, Paul C., Elizabeth T. Standring, and J. G. Brown. A bacterial necrosis of the giant cactus, *Carnegiea gigantea* (*Cereus giganteus*), has long been present in groves of that plant in southern Arizona. In recent years the disease has become more active and has killed many giant cacti. The distribution of the necrosis includes an area 200 miles by 100 miles in which the disease has actually been observed, but it probably involves a much larger territory. Only parenchymatous tissues of the cactus are destroyed. The bacterium concerned is here described for the first time.

Auxinic Activity of Leaves of Cabbage and of Myzus persicae Feeding on Them. Link, G. K. K., Virginia Eggers, and James E. Moulton. Ether extracts from leaves of cabbage and from *Myzus persicae* feeding on them were highly active in the Avena coleoptile test. After it was found that a second extraction following the initial one yielded active preparations, consecutive extracts were made at weekly intervals until the materials no longer yielded active substances. No cabbage-leaf sample yielded an active extract after the third treatment, whereas the aphid material still was active in the fifth extraction. In the first extraction the leaf extract was more active than that from the aphid, but the third aphid extract was more active than the first. The total activity of the 5 aphid extracts exceeded that of the 5 leaf extracts. The leaf extract in dilution, equivalent to 1 mg. of fresh leaf material, was active in the coleoptile test.

Microagglutination of Spores of Ceratostomella ulmi. May, Curtis, and John Zemlansky. Antiserum was prepared by injecting rabbits intravenously on alternate days with a total of 6 doses of 1, 2, 4, 6, 8, and 10 cc., respectively, of an 0.85 N saline suspension containing approximately 600,000 living spores of *Ceratostomella ulmi* per cc. Ten days after the final injection the rabbits were bled from the heart. The blood was stored overnight in a refrigerator. The clear supernatant serum was then decanted, the residue centrifuged, and a further small amount of clear serum recovered. The serum was then fractionated by standard methods and the globulin fraction obtained. This fraction was resuspended and then dialyzed until essentially salt-free, then filtered through a sterile Seitz filter, and stored in sterile vials. A drop of the globulin fraction was mixed on a microscope slide with a drop of spore suspension of *C. ulmi* in McIlvaine's phosphate buffer solution at pH 6.4. Agglutination was observed at once under the microscope when the spore concentration was 700,000 or higher per cc. From 2 to 20 spores were observed clinging together in varied positions. No similar agglutination was observed in the check suspensions to which no serum was added.

The Different Toxicity Ratings of Compounds by Different Fungi and Experiments in Spore-germination Tests of Fungicides. MCCALLAN, S. E. A., R. H. WELLMAN, AND FRANK WILCOXON. A study was made of 600 toxicity curves derived from 20 compounds tested against *Sclerotinia fructicola, Glomerella cingulata, Alternaria solani, Macrosporium sarcinaeforme, Botrytis cinerea,* and *Rhizopus nigricans* replicated in 6 experiments. In general, the LD50 values for any fungus or compound were equally reproducible. The fungi, in general, were equally sensitive, except *Rhizopus,* which was more sensitive. The fungi rated the compounds differently; *Sclerotinia* and *Macrosporium* were highly correlated and *Glomerella* and *Alternaria.* Four different types of slopes were observed on logarithmic probability paper: straight lines, curvature concave upwards, convex upwards, and sigmoid. Because of differences in slope, compounds will be rated differently at various LD levels. A "resistance level" of a particular lot of spores to all compounds could not be demonstrated, hence a standard will not correct experiment variation. Test fungi should be selected on the basis of reproducibility of results, ease of counting, and ready production of spores in quantity. An experimental design to give maximum efficiency is suggested: 5 doses, dose ratios of 2.000 or 1.414, 100 spores per count, no replicate counts, at least 2 fungi, graphic methods of obtaining LD50 and LD95, 2 replicate experiments and for error term highest interaction involving compounds.

Wetting Agents in Relation to the Control of Powdery Mildew of Roses under Greenhouse Conditions. MCCLELLAN, W. D. Experiments with spray materials for the control of powdery mildew of roses, grown under glass, have shown that the addition of certain wetting agents to spray solutions often increases the effectiveness of these spray solutions when a severe mildew infestation is present. Two factors, degree of wetting, as determined by contact angle measurements on rose leaflets, and toxicity, as measured by spore germination tests, have been found responsible for the differences between spray solutions containing different wetting agents. In one experiment, applications of Walcolized Sulphur 1: 1200 plus IN438 at concentrations of 1: 500, 1: 1000, 1: 2000, and 1: 4000 were compared. The percentages of lesions remaining active at the end of the experiment were 12.8, 16.7, 40.3 and 58.9 per cent, respectively. In another experiment applications of Walcolized Sulphur 1: 1200 plus IN438, IN181, Aresket 240, S.E.C. Oil, Santomerse S, Vatsol OT, Grasselli Sticker Spreader, each at a concentration of 1: 1000, and plus water alone were compared. The best control was obtained with Walcolized Sulphur plus Vatsol OT. Laboratory tests have shown that Vatsol OT wets rose leaflets better than any of the other materials tested and that it possesses considerable toxicity to the mildew conidia. Grasselli Sticker Spreader, IN438, and IN181 gave good control chiefly because of their toxicity to the mildew.

Control of Cercospora Leaf Spot of Peanut with Proprietary Sulphur Dust. MILLER, LAWRENCE, I. Studies initiated in 1938 were continued during 1939 and 1940 in Southeastern Virginia in an effort to evaluate the relative damage caused by Cercospora leaf spot and also to determine the effectiveness of different sulphur-dust-application schedules in the control of Cercospora leaf spot. Results of field experiments indicate that leaf spot causes approximately 30 per cent reduction in yield of nuts and 40 per cent reduction in yield of hay. Dusting 3 times with a conditioned 325-mesh sulphur reduced the amount of leaf spot to such an extent that excessive shedding of leaves was prevented. As a result, the sulphur-dusted peanut plants not only yielded more nuts and hay than nontreated plants, but also produced higher quality nuts and hay. It was demonstrated also that sulphur-dusted plants may be held in the ground 5 to 10 days longer than nontreated plants without serious loss of nuts through shedding.

Effect of Fungicides on Quality and Yield of Sour Cherries. MILLS, W. D., F. H. LEWIS, AND M. ADAMS. A five-year test on Montmorency cherries was initiated in Western New York in 1940. Four fungicides were applied to 4 rows each in a modified Latin square. Lime sulphur and Bordeaux mixture caused more foliage injury than flotation paste and copper oxychloride. The number of cherries per pound at harvest with lime sulphur (124) and Bordeaux mixture (121) was significant over flotation (111) and copper oxychloride (106). The yield in pounds per tree was significantly less with lime sulphur (34) and Bordeaux (33) than with flotation (44) and copper oxychloride (41). The percentage of solids in solution in the fruit was significantly higher for Bordeaux (16.2) and copper oxychloride (15.4) than for lime sulphur (14.2) and flotation (13.7). Coccomyces spot was low for all treatments through July. On August 23 leaf spot on flotation was 23, lime sulphur 10, copper oxychloride 3, and Bordeaux 1 per cent. Total retained leaves August 23 were 93–96 per cent with all treatments. On October 1 significantly more were retained on Bordeaux (90) and copper oxychloride (89) than on flotation (76) and lime sulphur (69) per cent. Significant correlations were found between spray injury and size of cherries and between cherry size and yield.

An Undescribed Storage Rot of Celery. NEWHALL, A. G. A serious storage rot of late celery, causing losses in 5 counties in western New York, has been traced to a fungus described by Westerdijk in 1924 as *Cercospora cari,* the cause of "Anthracnose" on caraway. The name "greenish black rot" is suggested to distinguish this disease from the Phoma root rot with which it has been confused. Infection seems to come from the soil, although first symptoms rarely appear until after 8 or 9 weeks in cold storage. A soft rot then present progresses rapidly upward on the butt ends. Of 12 commercial varieties tested on infested muckland none was markedly resistant, though Easy Bleaching, Utah, and Columbia were somewhat less affected than Giant Pascal, Golden Plume, and Meische's Improved Golden. The fungus is characterized by coarse, deeply colored, torulose mycelium and sparsely produced hyaline to subhyaline, falcate conidia possessing an appendage on the basal cell that really excludes it from the genus *Cercospora.* Growth in culture takes place between 0° and 27° C. with the optimum close to 15°, and at a pH range between 3.35 and 7.32 with an optimum near 5.3. Of 10 fungicidal dips applied at harvest time to the butt ends of several hundred celery plants only organic mercurials, considered unsafe, were effective in reducing subsequent storage rot.

Host Range and Overwintering of the Lettuce Shot-hole or Anthracnose Fungus, Marssonina panattoniana. NEWHALL, A. G. Anthracnose, shot hole, or ring spot of leaf and head lettuce occurs sporadically in market-garden areas in New York State on early spring and late fall plantings out-of-doors, whence it makes its way into greenhouses. By inoculating with spore suspensions the disease has readily been transferred from cultivated lettuce, *Lactuca sativa* to the following native species, *L. scariola, L. scariola* var. *integrata, L. spicata,* and *L. canadensis.* In addition the following species sent by Ross C. Thompson also have been infected in the seedling stage, *L. altaica, L. laciniata, L. saligna, L. virosa, L. indica, L. graminifolia,* and *L. muralis.* Soil from beneath diseased plants was found harboring the disease for 3 months but not for 6, at which latter time it had become air-dry. Infected plants of *L. scariola* and its variety *integrata* with abundant stem lesions have been found growing wild in 2 counties. Lettuce seedlings have been infected with the fungus from this source indicating the probability that *Marssonina panattoniana* overwinters on native Lactucas, particularly *L. scariola* var. *integrata* in New York.

Silver Sprays with Promising Fungicidal and Adhesive Properties. NIELSEN, L. W. Over 70 silver preparations were tested for fungicidal and adhesive properties by the following method: potted potato plants were sprayed, given an artificial-rain treatment and then inoculated with *Phytophthora infestans.* The number of lesions developing on the plants, with and without rain, was taken as a measure of the adhesive and fungicidal properties, respectively. Three mixtures offering special promise contained silver nitrate, calcium hydroxide and a third component, *i.e.,* manganous sulphate, ferrous sulphate or ferric sulphate. In each case the molar concentration of the third component giving maximum practical adhesiveness was equal to that of the silver nitrate, *i.e.,* 0.0035 molecular weight per gallon. This concentration equals 100 p.p.m. of silver. For the mixtures containing either ferrous or manganous sulphate, a calcium hydroxide concentration 1.1 times the sum of the chemical equivalents of the metal salts gave greatest practical adhesiveness. For the ferric sulphate mixture, a calcium hydroxide concentration approximately 5 times the sum of the chemical equivalents of the metal salts is most effective. The most adhesive spray is the ferrous sulphate mixture. These sprays have compared favorably with Bordeaux mixture in various tests.

The Development of a New Method for Determining the Effect of Supplements upon the Solubility of Copper Fungicides. NIKITIN, A. A. Supplements may change the solubility of fungicides and their toxicity. It is essential to determine this change in the solubility of fungicides. Changes in solubility, caused by supplements, are not always pronounced enough to permit their measurement. It is necessary to amplify the change in solubility that may be caused by the supplements. Organic acids are not practical, since they change the chemical composition of fungicides. Apple juices increase the solubility of copper fungicides to the desired extent without altering their chemical composition. This new method of measuring action of supplements on solubility of fungicides is based on the determination of soluble copper formed in the presence of fruit juices. The effect of supplements at higher concentrations of copper ions is more pronounced, and the study of the chemical compatibility of supplements with fungicides is facilitated. Wheat flour activates copper fungicides less than soya flour. This was confirmed by field tests on apples, where the use of soya flour caused more injury than wheat flour. A study was made on the degree of dissolving action of juices from several varieties of apples in relation to their susceptibility to spray injury.

Control of Club Root on Cabbage with Mercurials. PALMER, RALPH G. Mercuric chloride, dissolved in water (1–100 and 1–1500) and mercurous chloride suspended in water at the rates of 5 and 7.5 lb. per acre, gave striking control of club root on cabbage. The mercuric chloride was used at the dilutions specified above, and the mercurous chloride was suspended in water with the aid of gum arabic. One-third pint of the various solutions was applied to each plant at planting time by means of the usual watering attachment on the planter. The plots were located on sandy soil known to be heavily infested with *Plosmodiophora brassicae.* Roots of check plants were severely clubbed and deformed and lateral fibrous roots were almost entirely absent. Treated plants had normal root systems with a few lateral roots showing clubbed portions. Check rows, treated only with water, failed to mature more than 10 per cent of marketable-size heads, and many of the plants died in midsummer, particularly in the wetter portions of the field. All treatments gave a very large percentage of mature and marketable heads, even in moist locations.

Effects of Potash on the Tip Burn of Cabbage. PALMER, RALPH G. Increase during recent years in prevalence and severity of tip burn on cabbage prompted experiments testing the relation of potash and phosphorus to this nutritional disorder. Trials of broadcast applications of 1515 lb. per acre of a home mixed $4\frac{1}{2}$–13–12 fertilizer, of the same home mixture, less the potash, and of 1000 lb. per acre of a factory mixed 5–10–5 gave decided differences in tip burn and yield on sandy soil in Monroe County. The plots receiving potash in either mixture were almost completely free from tip burn, even in the lower portions of the field, and what slight tip burn was found apparently did not reduce yields or quality of the affected heads. Where no potash was used, nearly every plant showed injury, the plots averaging 64 per cent moderate to severe tip burn. The differences at the plot margins were most striking. Yields were definitely reduced on the no-potash plots, averaging 14.97 tons an acre as compared with 23.29 tons an acre on the home mixed and 24.16 tons on the factory mixed complete fertilizers. Differences in tip burn and yield were fully consistent between the various replications.

Treating Chlorotic Pin Oaks by Trunk Injections and Soil Treatments. PIRONE, P. P. Inserting collodion capsules containing ferric phosphate in holes bored into trunks of chlorotic pin oaks restored normal green color to foliage and increased twig growth within a few months after treatment. The trees were growing along streets in Newark, New Jersey, in an area underlaid with ashes and other refuse, the entire area having previously been a dump. Five grams of ferric phosphate per inch-diameter of the trunk were used. The number of holes ($\frac{1}{2}$ inch diameter) varied from 1 to 4 per tree, depending on trunk diameter. The chemical was inserted on April 24, 1940, when the trees were still dormant. The holes were plugged with grafting wax. Besides adequate control trees, the tests included soil treatments. A 3- to 5-lb. mixture of powdered sulphur, aluminum sulphate, and ferrous sulphate (ratio of $\frac{1}{2}$–$\frac{1}{4}$–$\frac{1}{4}$ per lb.) was placed in holes around the trees according to standard fertilizing procedure for shade trees. Three months later, the leaves of most trees treated by trunk injections with ferric phosphate were normal-green, whereas nearby nontreated trees showed severe chlorosis, as in previous years. Trees receiving soil treatments showed considerable improvement in leaf color and twig growth over nontreated trees but were less healthy than those receiving trunk injections. In all, more than 30 trees varying from 2 to 8 inches DBH were involved in the tests.

The Mode of Overwintering of Entomosporium maculatum in Louisiana. PLAKIDAS, A. G. In recent years *Entomosporium maculatum* leaf spot has become serious on the Chinese sand pears in Louisiana, causing severe defoliation in early summer. For years it was evident that the dead leaves served as the source of inoculum because infection in the spring was noted to start on the branches close to the ground and gradually spread upward. It was assumed that ascospores developed on the dead leaves, but, since Smith and Goldsworthy had failed to find the *Fabraea* stage in Maryland and Missouri, it appeared doubtful that it would occur in Louisiana. Dead leaves were examined periodically during the winter and spring in 1939 and 1940. No ascospores were found, but conidia were found in great abundance on the overwintered dead leaves in the spring. Infection was secured by placing dead leaves in cheesecloth bags on leafing pear branches in early spring. Bark cankers were not found, and eradicative sprays (4 lb. bluestone, 4 lb. lime, 2 lb. of monocalcium arsenite, or iron arsenite, 1 qt. fish oil, 50 gal. water) applied late in January and designed to inactivate any cankers that might be present, gave no control. It is concluded that, in Louisiana, the fungus overwinters in the mycelial stage on the dead leaves and produces conidia in the spring.

Dutch Elm Disease Fungus Prevalent in Bark-beetle-infested Elm Wood. RANKIN, W. HOWARD, K. G. PARKER, AND DONALD L. COLLINS. Samples of elm wood infested by the overwintering brood of *Scolytus multistriatus* were collected at random from stations about 5 miles apart over an area of approximately 3000 square miles where the Dutch elm disease has existed for two or more years in New York State. By daily culturing of a fraction of the adult Scolytus beetles emerging from the 154 samples, beetles carrying *Ceratostomella ulmi* were obtained from 59 of the samples or 38 per cent of the total number. The samples were representative of over 100,000 dead and dying elm trees and additional elm wood on the ground found during the past year at over 30,000 locations in this same area. The average annual incidence of elms found dying of the Dutch elm disease in this area is only about one in three square miles. This proof of the prevalence of sources of inoculum and the low incidence of elms killed by the fungus seemingly indicate either a low efficiency for the beetle as an inoculating agent or a critical balance requisite for infection that is rarely satisfied.

A New Virosis of Peach in Utah, Resembling X-disease (yellow-red Virosis). RICHARDS, B. L. AND LEE M. HUTCHINS. Symptoms resembling in some but not all respects the X-disease first described from Connecticut in peach and chokecherry (*Prunus virginiana*) were first observed in northern Utah in western chokecherry (*Prunus demissa*) in 1937, and in peach in July, 1939. Prevalence has increased in both species. Incidence of infection in peach orchards varied from a trace to 80 per cent of the trees in 1940, the highest incidence being in the older trees. Artificial transmission of the disease from peach to peach was secured by graft inoculations performed in 1939 and 1940, the incubation period being variable. Of 40 Elberta nursery trees inoculated on July 26, 1940, all trees developed the disease within 6 weeks. Intercommunicability of the disease between western chokecherry and peach has not been established but is under investigation. In Utah, survey data show that affected plants of western chokecherry are present in the same general district where the disease is found in peach. However, these distribution studies show definitely that incidence of infection may be high in orchards located 1 mile or more from affected western chokecherry, and it is believed that under conditions in this area the disease spreads freely from peach to peach.

Growth Substance in Crown Gall Related to Time After Inoculation, Critical Temperature, and Diffusion. RIKER, A. J., BERCH, HENRY, AND B. M. DUGGAR. Some aspects of the role of auxins in crown-gall development on tomato have been studied. Estimations of auxin quantities were obtained through more than 7,000 readings with the Avena coleoptile test. About 90 per cent of the day-to-day variations in the Avena test, as employed, or eliminated by converting curvature values to heteroauxin equivalents. No significant differences in auxin content were found between inoculated and control tissues at 1, 4, 8, and 16 days after inoculation. Galls and control stems from decapitated plants contained about half as much auxin, respectively, as those from whole plants. No significant difference was found between the auxin content of inoculated tomato stems grown at 27° C., where galls develop, and at 31° C., where galls do not develop. No significant difference appeared in the amount of auxin diffusing from stem segments bearing galls and from control segments. These 3 lines of evidence show that crown-gall and control tissues are similar in relation to auxin obtained by extraction and diffusion. Consequently, it seems that auxins are not primarily responsible for crown-gall formation.

Ascospores of Ceratostomella ips and Ceratostomella montium. RUMBOLD, CAROLINE T. The ascospores of the blue-stain fungi *Ceratostomella ips* and *C. montium* are rectangular parallelepipedal with square ends, possessing flanges on the outer walls. In water the spores appear ellipsoidal or cylindrical because of shape of the protoplasm. When stained with certain dyes the flanges may be seen readily. In the strain of *C. montium* studied the initial ascogenous hyphae grow among the large hyaline cells in the cavity of the immature perithecium, which is lysigenic. The hyaline cells disintegrate and fill the cavity with a mass of predigested food in which grow young asci containing 1, 2, 4, and 8 nuclei. The asci become detached and disintegrate before the ascospores mature. At first the ascospores are globular, then ellipsoidal, then rectangular parallelepipedal. Their shape does not result from pressure within the perithecial cavity.

Linkage in Rice of Two Resistant Factors to Cercospora oryzae. RYKER, T. C. The rice variety Blue Rose, which is susceptible to race 1 of *Cercospora oryzae*, but moderately resistant to race 2, was crossed with Blue Rose selection 2854–3, which is resistant to race 1, but susceptible to race 2. Reciprocal crosses were made and 26 F_1 plants were grown. From these a total of 1899 F_2 plants were tested for their reaction to race 1, to race 2, and to both. Seed of 89 F_2 plants representing 7 families was planted in the greenhouse and the plants were tested for their reaction to both races of the fungus. Also, 54 F_2 plants were tested by growing their progeny in the field and inoculating with race 2.

The F_2 population segregated with approximately 3 plants resistant to 1 susceptible to each race of the fungus. The plants susceptible to one race of the fungus were, with few exceptions, resistant to the other. When the F_3 plants were tested it was found that F_2 plants, susceptible to race 1, were homozygous for resistance to race 2 and plants, susceptible to race 2, were homozygous for resistance to race 1. Furthermore, F_2 plants, resistant to both races, proved to be heterozygous for resistance to each race. This suggests 2 single linked factors for resistance to races 1 and 2. In only a few instances was there any indication of cross-overs. These have not yet been tested.

Development of Diplodia zeae and Gibberella saubinetii in Maize Pith Following Stalk Inoculations. SEMENIUK, G. In 1938 and 1939, respectively, *Diplodia zeae* spread 78.1 and 68.0 per cent more in hybrid than in inbred strains of maize, while *Gibberella saubinetii* spread 4.5 and 55.4 per cent more. *D. zeae* spread 59.2 and 172.5 per cent more than *G. saubinetii* in the pith of inbred lines and hybrids, respectively, in 1938 and 80.7 and 113.5 per cent more in 1939. The length of the inoculated internode bore no relationship to the spread of the pathogens. Correlation coefficients (r) in the spread of the pathogens in the pith of inbred lines between 1938 and 1939 were highly significant for *D. zeae* ($r = +0.413$; 1% point $= 0.356$) and *G. saubinetii* ($r = +0.578$; 1% point $= 0.356$). The spread of *D. zeae* and *G. saubinetii* in the pith of inbred lines in 1938 ($r = +0.432$; 1% point $= 0.335$) and in 1939 ($r = +0.652$; 1% point $= 0.269$) also were highly correlated. With hybrids, however, nonsignificant r values were obtained in 1938 ($r = -0.177$; 5% point $= 0.463$) and in 1939 ($r = -0.067$; 5% point $= 0.526$). The extent of natural stalk rotting of maize caused by *D. zeae* was nonsignificantly correlated in 1939 with *D. zeae* development in the pith following stalk inoculation of inbred lines ($r = +0.191$; 5% point $= 0.206$) and hybrid strains ($r = +0.076$; 5% point $= 0.413$). This same lack of relationship was obtained in 1940.

An Eradicant Spray for the Control of Apple Scab and Raspberry Anthracnose in Minnesota. SHARVELLE, E. G. In 1937–1940 several States reported successful control of apple scab by "floor-spraying" with the sodium salt of dinitrocresol (Elgetol). In the 1940 season 4 rows in an experimental McIntosh orchard were treated in early spring with a ground spray of 0.5 per cent of this material at the rate of 500 gallons an acre. A regular spray schedule was applied to the entire orchard during the summer. In the fall 1000 random-selected apples from trees in the "floor sprayed" rows were hand graded for scab. A comparable sample was taken from 4 control row at the other side of the orchard. From the non-floor-sprayed plot, 20.5 per cent of the fruit was infected with scab as compared to 4.1 per cent from the plot that did receive an application of Elgetol. In addition to significantly reducing the amount of infected fruit, the severity of infection as denoted by size and number of scab lesions was markedly reduced by the use of Elgetol as a "floor-spray." Preliminary experiments, with 0.5 per cent Elgetol as a delayed dormant spray, indicated that this material is worthy of further investigation as an eradicant spray for raspberry anthracnose. Infection was reduced from 100 per cent of the hills infected in the nonsprayed rows to 80 per cent where Elgetol was used as a delayed dormant spray, severity of infection also being greatly reduced by the spray.

A Preliminary Investigation of the Value of a New Seed Protectant for Canning Peas in Minnesota. SHARVELLE, E. G., AND B. F. SHEMA. During the past few years the common root rots of peas, caused mainly by *Rhizoctonia*, *Pythium*, and *Aphanomyces*, have become a factor of some concern to the Minnesota canners. Attempts have been made in the past to combat these troubles by the use of seed protectants with inconclusive results. During the past summer, a new, organic, non-metallic seed protectant, "Spergon," was tested on canning peas at 7 localities in Minnesota. Spergon was applied as a dust to the seed at the rate of 2 oz. per bu., and these dusted seeds were planted in 7 1-acre experimental plots in cooperation with 7 canneries in southern Minnesota. The maximum increase from the use of Spergon amounted to 800 lb. of green peas per acre, the average increase being 350 lb., or approximately 14 per cent. Preliminary investigations indicate that Spergon shows promise as a seed protectant for canning peas in Minnesota, particularly on early sweet varieties. Besides its seeming value as a seed protectant, Spergon appears to stimulate the growth of the resulting plants.

Critical Tests with Crop Rotations for Control of Granville Wilt of Tobacco. SMITH, T. E. These experiments were carried out on soil demonstrated by prior cropping to be highly infested with *Bacterium solanacearum*. Fourteen crops or combinations were grown in 2-, 3-, 4-, or 5-year rotations with tobacco. Corn, soybeans, red top, cotton, crab grass, cowpeas, lespedeza, crotalaria, and sorghum gave wilt control but were not consistently effective. Sweet potatoes, weed fallow and bare fallow were comparatively ineffective. On small plots carefully protected from reinfestation, corn was not significantly more effective than soybeans, both were more effective than bare fallow, and all three cropping treatments were more effective than continuous tobacco. Within the same season 5-year rotations were more effective than 2- or 3-year rotations. The difference between 2- and 3-year rotations was not significant. Absence of wilt-susceptible plants during the years intervening between tobacco crops did not assure a high degree of wilt control. Four- or 5-year rotations consisting of corn, soybeans, cotton, red top, small grains, or lespedeza, appear best for the usual farm conditions. The results varied widely from season to season and confirmed farmers' experience that crop rotation fails to give consistent results.

Successful Cross-Inoculations with Fusarium from Tobacco and Sweet Potato. SMITH, T. E., AND K. J. SHAW. Fusarium wilt of tobacco appeared following sweet potatoes at Creedmoor and McCullers, North Carolina, Florence, South Carolina, and Tifton, Georgia. At the first 3 locations this was the first known appearance of the disease in the immediate areas, and, at the fourth location, first appearance was associated with both continuous tobacco and sweet-potato rotations. No Fusarium wilt developed on tobacco grown in rotations with cotton and cowpeas, although in some instances Fusarium infection exceeded 50 per cent on these crops. On plants grown in sand culture in the greenhouse, successful inoculations on sweet potatoes were made with 21 Fusarium cultures from 21 naturally infected tobacco plants collected in Maryland, North Carolina, South Carolina, and Georgia. Tobacco was slightly more susceptible than the sweet potato. Several collections from sweet potato were pathogenic on tobacco, but these were collected in areas where *Fusarium* was present on tobacco.

Susceptibility of Planera and Several Elm Species to Ceratostomella ulmi. SMUCKER, S. J. The susceptibility of several species and varieties of elm to *Ceratostomella ulmi* was tested in 1940. Results, based on extent of dieback of the main leader at the end of the growing season, showed *Ulmus laevis, U. procera, U. japonica* and the "Mormon elm" less susceptible than *U. americana* and *U. foliacea; U. foliacea* var. *Christine Buisman,* highly resistant; and *U. pumila* and its varieties *pinnato-ramosa* and *arborea,* slightly susceptible. Three trees of the water elm, *Planera aquatica,* a new host genus for *C. ulmi,* developed pronounced wilt and considerable dieback shortly after inoculation with *C. ulmi.*

Effect of Nitrogen Nutrition on Virus Inactivation in Tobacco. SPENCER, ERNEST L. Experiments have shown that tobacco-mosaic virus (*Marmor tabaci*) can be partially inactivated within the host by reducing the nitrogen supply. Turkish tobacco seedlings (*Nicotiana tabacum*) in sand cultures were inoculated with tobacco-mosaic virus. Ten days later the plants were divided into 2 groups; one group continued to receive a plus-nitrogen solution, but the other group was supplied a minus-nitrogen solution. At 4-day intervals thereafter, representative plants from each group were harvested, frozen at -10° C. for 24 hours, and then minced. The expressed juice was assayed for total-protein content, virus-protein content, and relative virus activity. The virus protein was isolated in an ultracentrifuge, measured, and then tested for virus activity. In nitrogen-deficient plants the virus-protein content, as well as the plant-protein content, remains practically constant, whereas in nitrogen-fed plants each increases more than 5 times during a 16-day period. Hence, neither the plant nor the virus appears able to utilize the protein of the other when nitrogen is deficient. Nevertheless, th virus protein is altered in some way with a resultant decrease in activity.

Accuracy of the Local Lesion Method for Measuring Virus Activity. SPENCER, ERNEST L., AND W. C. PRICE. The accuracy of the local-lesion method for measuring activity of tobacco-mosaic virus (*Marmor tabaci*), alfalfa-mosaic virus (*M. medicaginis*), tobacco-necrosis virus (*M. lethale*), and tobacco-ringspot virus (*Annulus tabaci*) has been investigated. The method used is based on a comparison of the dilution curves obtained with an equal number of dilutions (usually 3) of both the standard and the unknown tested on an equivalent number of leaves of the test plant, *Phaseolus vulgaris* var. Early Golden Cluster. The data show that this comparison is more accurate than that involving only one dilution of the standard and one of the unknown. The error of estimation of tobacco-mosaic virus, for example, was less than 15 per cent when the difference between the standard and the unknown was 10 per cent, less than 30 per cent when the difference was 25 per cent, and about 50 per cent when the difference was 75 per cent or more. The method should prove applicable to other plant viruses, provided a test plant that responds to infection by production of suitable local lesions is available.

The Growth of Basisporium gallarum in Maize Cobs. STANDEN, J. H. Three conditions seem necessary to the growth of *Basisporium* in maize cobs: dying or dead cob tissues; loose, spongy cob tissues usually associated with immaturity; and conditions of temperature and moisture favorable to growth of fungus. Investigations dealt mainly with resistance and susceptibility as related to cob structure. Chemical analyses of cobs readily attacked by *Basisporium* show sugar content of such cobs higher than that of more mature cobs. However, addition of sugar and peptone to meal of well-matured cobs failed to produce growth of fungus corresponding to that on meal of immature cobs. Substances other than simple nutrients necessary for growth of the fungus apparently are more abundant in immature than in mature cobs. On addition of extract of immature to well-matured cobs, growth of *Basisporium* was more vigorous than on cobs not containing the extract, but the retting produced by the action of the fungus on immature cobs was not obtained. In general, immature, soft cobs were susceptible; mature, hard cobs were resistant. Lignin fractions were only slightly higher in the latter, but specific gravity was higher and water-holding capacity lower, indicating a difference in tissue structure. Therefore, both composition and structure of maize cobs were associated with resistance and susceptibility to *Basisporium gallarum.*

A Comparative Study of the Chemical Composition and Calorific Value of Decayed Cordwood. Steinmetz, F. H., and M. T. Hilborn. Previously reported studies showed that the calories per gram of decaying wood decreased faster than the decrease in specific gravity, indicating that wood components are not alike in calorific value. A chemical analysis of samples of decaying wood shows, however, that all the principal wood components decrease similarly as decay progresses.

A Transmissible Mosaic of American Elm. Swingle, R. U., P. E. Tilford, and Charles F. Irish. A mottled-leaf condition resembling a virosis was observed on American elm in the vicinity of Cleveland, Ohio, in 1927. Later, trees showing similar symptoms were noted in several locations in Ohio and other eastern States. Usually some leaves on diseased trees are normal in size and texture, whereas others on the same tree may be abnormally large or small, stiff, and frequently distorted. Small leaves show typical yellow and green mottling accompanied by rugosity. On some trees the mottling is more diffuse. Mild to moderate brooming of branches may occur. There is no discoloration of bark or wood in either tops or roots. Wood of affected trees seems dry and is often brittle. Diseased trees generally decline in vigor from year to year becoming unsightly because of thinning of foliage and death of scattered branches. Thus far, none of the diseased trees under observation has died. In June, 1939, 22 healthy trees were grafted with diseased bark patches secured from 4 mosaic elms at Cleveland, Ohio. In June, 1940, 19 of these trees showed typical mosaic symptoms. All check trees and those on which grafts were unsuccessfully attempted remained healthy.

Copper Fungicides for Control of Cherry Leaf Spot. Taylor, Carlton F. Spray experiments in West Virginia, in 1940, showed that 4 lime-sulphur sprays inadequately controlled leaf spot (*Coccomyces*) on Montmorency cherries. Bordeaux mixture at 3–5–100 and at 2–4–100 adequately controlled leaf spot, but, in some cases, caused excessive leaf yellowing and defoliation. Certain proprietary copper compounds were much superior in preventing late-season defoliation. They offered adequate protection and caused very little leaf yellowing. In limited trials copper oxalate (4 lb. of 19 per cent metallic-copper content, and 3 lb. hydrated lime per 100 gall. water) gave adequate protection and caused notably little foliage yellowing. Certain copper sprays, applied August 1 (immediately after harvest), to severely infested trees reduced subsequent infection and defoliation. On the copper-sprayed trees much of the foliage present at this date was retained until November, while those sprayed with lime sulphur on the same date were virtually defoliated by mid-September.

Occurrence, Identification and Species Integrity of the Loose Smuts of Barley Ustilago nuda, U. nigra, and U. medians. Tapke, V. F. Five hundred barley loose-smut collection from many parts of the country were studied. On potato-dextrose agar at 20° C. the spores of the various collections produced: (1) only the mycelial threads that characterize *U. nuda* (Jens.) Kell. and Sw., or (2) only the sporidia that characterize *U. nigra* (Tapke), or (3) some spores produced threads while the remainder produced sporidia. The last is the mixed type on which *U. medians* (Biedenkopf) was based when originally described in 1894. By 3 different techniques it was conclusively proved that every "*U. medians*" collection was simply a mixture of the mycelium-producing *U. nuda* and the sporidia-producing *U. nigra*. *U. medians* evidently does not exist in the United States or elsewhere and apparently was erroneously based on a mixture of 2 smuts. *Ustilage nuda* and *U. nigra* are similar in appearance but require widely different seed-treatments for control. These studies show that a simple spore-germination test may be relied upon for identifying *U. nuda* and *U. nigra* and for making sound recommendations for seed treatments for control.

A Proposed System of Virus Nomenclature and Classification. Thornberry, H. H. The herein proposed system of virus nomenclature and classification is based upon the infectious intracellular agents that are parasitic on the generally recognized groups of organisms. Technical names of the viruses based upon their chemical constituents and the Geneva system is impossible at present. Therefore, and for the purpose of utility, the intracellular infectious agents that fail to increase in a cell-free medium are assigned to an Order (*Biovirales*) in adjunct to the bacteria (Class *Schizomyceta*, Phylum *Thallophyta* of the PLANT KINGDOM). The families 1. *Rickettsiaceae* Pinkerton 1936 (genera *Dermacentroxenus* Wollbach 1919, *Rickettsia* Pinkerton 1936, and *Wollbachia* Hertig 1936) 2. *Phytoviraceae* (genera *Phytovirus, Pteridovirus, Bryovirus, Thallovirus,* and *Bacteriophagus*) and 3. *Zooviraceae* (genera *Zoovirus* and *Insectivirus*) are proposed. Classification of the *Phytovira* is based upon susceptible groups of organisms, methods of transmission, and symptoms on the respective standard hosts. The species portion of the binomials for *Phytovirus* is compounded from the first, or more, syllables of the generic name of an important host, prefixed to a Latin word implying one of the chief symptoms on a respective standard host (*Phytovirus nicomosaicum* variety *vulgare*). Six rules of proof for a virus disease of plants are suggested.

Current Research in the Control of Injurious Rodents. U. S. Fish and Wild Life Service and Kenneth J. Kadow. Rodent damage to root stocks, bulbs, and tubers by breaking protective surfaces and lowering the vitality of the plant prepare the way for pathological conditions. In orchards and nurseries throughout Delaware root injury is prevalent due to activity of meadow and pine mice. Types of injury are illustrated. New control methods using fruit and vegetable habits are explained. The value of soil fumigants and repellent coatings to offset rabbit damage and damage by mice and moles is discussed.

Experimental Production of Symptoms in So-called Recovered Ringspot Tobacco Plants and Its Bearing on Acquired Immunity. Valleau, W. D. The effect of lowering the greenhouse temperature on tobacco plants inoculated with 6 distinct seed-transmitted strains of ringspot virus (*Annulus tabaci*) was studied. Affected seedlings of 4 strains were yellow, a fifth bleached so-called recovered leaves slightly, the sixth was non-bleaching. Following so-called recovery of inoculated plants, growing-point leaves developed marked chlorosis and necrosis (distinct from bleaching) each time the temperature was lowered to 20° C., except ''yellow ringspot'' plants. With a rise in temperature diseased leaf growth was more normal and new leaves appeared normal except for bleaching, which occurred, as the leaves became older, on plants affected by 5 strains. Pollen sterility varied from slight (''yellow ringspot'') to almost complete in other strains, resulting in a corresponding reduction in seed set. The results are considered as proof that ringspot-affected plants do not recover, but merely enter a second phase of the disease following invasion of embryonic tissue. Plants in the second phase cannot be considered to have developed immunity, according to the usually accepted definition of the word. Protection afforded by one strain of the virus against reinfection with the same or another strain can be explained by competition.

Studies on Boron Deficiency in Garden Beet (Beta vulgaris). Walker, J. C., and J. P. Jolivette. The development of internal black spot, due to boron deficiency, was found to vary from 79 per cent diseased plants in variety Good for All to practically none in Half Long Blood; strains of Detroit Dark Red, Crosby Egyptian, and Early Wonder ranged between these extremes. Garden beets are, in general, more susceptible than sugar beets. Where 20 lb. of borax per acre was sufficient to completely control heart rot of the latter, 40 lb. per acre was necessary for commercially successful control of internal black spot of garden beet. The disease was reduced to practically nil when borax was applied as a spray at 40 pounds per acre in midseason up to 1 week before top symptoms appeared. When a 40-lb. treatment was made on Poygan clayey silt loam (pH 7.5–7–8) the corrective effect still prevailed the second successive season with garden beets. When a 20-lb. treatment was made in the same field the treatment was effective the second season with sugar beets, but it was not completely effective during the third season.

Transmission of the Planetree Ceratostomella in Asphalt Wound Dressings. Walter, James M., and Paul V. Mook. Field observations on cases on the Ceratostomella canker and wood-stain disease of sycamore, in which infection obviously began at pruning wounds treated with asphalt paints, indicated that the latter probably were not antiseptic and might transmit the fungus. Arborists, for practical reasons, so handle their paint cans and brushes that sawdust and bark fragments are carried by them from wound to wound. Agar-plate tests of the survival of the fungus in wound dressings were made, the inoculum being infected sawdust. The results were positive for a number of the popular asphalt type. Two kinds of dressings that prevented growth of the fungus were found to be toxic to sycamore cambium. Experimental applications of 12 brands of wound dressings containing Ceratostomella-infected sawdust were made to pruning wounds on London planes. Infections occurred with 7 asphalt dressings, the percentage of treated wounds developing the disease varying from 36 to 100. This information suggests the need for antiseptic wound dressings that are non-injurious to cambium. Further study of the problem, involving tests of the effects of fungicides added to asphalt paints, is in progress. The planetree-disease demonstration includes materials illustrating survival and transmission of the fungus in asphalt dressings.

The Relation of Bacterium malvacearum to Anthracnose Boll Rot of Cotton. WEINDLING, RICHARD, AND PAUL R. MILLER. During surveys of cotton-boll rots in 1938–1940, *Glomerella gossypii* was isolated frequently from boll lesions bordered by water-soaked green margins. In 1940, *Bacterium malvacearum* was secured from the margin of such lesions in most cases when isolation was attempted. In order to determine more accurately the relation of the bacterium to anthracnose boll rot, flowers and young bolls of cotton were atomized with suspensions of *B. malvacearum* and of conidia of *Glomerella*. The organisms were used singly and in mixture. Anthracnose lesions resulting from inoculation with the mixture were more conspicuous and much more numerous than those produced by the fungus alone. Furthermore, only infection with the mixture produced the kind of lesions with water-soaked margins noticed in the surveys. Evidently, infection of bolls with *G. gossypii* is frequently made possible by *B. malvacearum*.

Duration of Inhibition of Spore Germination: A Factor in Spore-germination Tests of Fungicides. WELLMAN, R. H., AND S. E. A. MCCALLAN. Duration of inhibition, as measured by time lapse before determining percentage of germinated spores on glass slides in triplicate experiments, was not the same for 6 compounds examined (copper sulphate, zinc chloride, Bordeaux mixture, Cuprocide and two aliphatic organic compounds) on a single fungus or for a single compound on 4 fungi examined (*Sclerotinia fructicola, Alternaria solani, Glomerella cingulata,* and *Macrosporium sarcinaeforme*). In some cases, no significant change in percentage germination could be demonstrated after 6 hours; in others, the change in percentage germination between 24 and 48 hours was significant. Since no significant change took place between 48 and 96 hours, little additional information can be obtained by counts made after 48 hours. The reproductibility of results did not differ at any given time of counting between 6 and 96 hours. In order to compare toxicities of compounds it is necessary to consider time lapse before counting, as well as the concentration of the chemical used. This may be done by making counts at times varying in a logarithmic progression.

A New Turf Disease. WERNHAM, C. C., AND R. S. KIRBY. Greens keepers and superintendents of golf courses in Pennsylvania and certain other States are concerned about the thinning-out of Metropolitan bent during hot weather. Laboratory isolation, followed by subsequent inoculation studies, has shown this condition traceable to a species, or perhaps 2 species, of either *Helminthosporium* or *Curvularia*. Further field and laboratory observation has shown that the sudden disappearance of *Poa annua*, spoken of as the ''going-out'' or ''melting-out,'' is attributable to the same causal organism as that concerned with Metropolitan bent. Single-spore isolates of the fungus, reveal an optimum growth at 30° C. with a rapid falling off below 25°. These data substantiate the field observation, that the disease is severe only during hot weather. Infected *Poa annua* is killed under field conditions, while Metropolitan bent recuperates in cool fall weather. Experiments to control the disease on *Poa annua* in the fairways indicate that small applications of dusting sulphur or zinc oxide are the most promising treatments. Watering the fairways checked the disease at first, but, later, intensified it. Field data indicate that certain selected strains of *Agrostis palustris* are resistant.

Phomopsis Fruit Rot of Tomato. WIANT, JAMES S. A species of *Phomopsis* was found causing a fruit rot of Texas tomatoes received at the New York market in mid-July, 1940. Watersoaking and rapid softening of the flesh, followed by a browning of affected areas, are characteristic symptoms on both green and ripe fruits. The optimum temperature for progress of the decay and for growth of the pathogen in culture is near 85° F. Although greatly retarded at 40° F., both continued slowly at that temperature. A comparison of the tomato pathogen with several species of *Phomopsis* responsible for market decays of other commodities is being made by cross-inoculation and cultural methods.

Varietal Resistance of Wheat to Loose Smut. WINGARD, S. A. Studies of 80 varieties and selections of wheat over the past 20 years indicate a marked difference in their relative susceptibility to loose-smut infection. Of the 45 commercial varieties and selections tested, Forward (C.I. 6691), Kawvale (C.I. 8180), Leapland, Leap (C.I. 6958), Marmin (C.I. 11,502), Pete Edwards, Redhart, Tenmarq, Sel. T–47–55, Trumbull (C.I. 5657), Fulhio (C.I. 6999), and Fulcaster (C.I. 6471) over a 5-year period have been consistently low in loose smut; whereas Fultz, Gladden (C.I. 5644), Michigan Amber (C.I. 5620), Arlando (C.I. 10069), Purdue No. 1 (C.I. 11380), Poole (C.I. 3488), Red Rock (C.I. 6951), and T–N–1047–Ohio have been consistently high in loose smut. Of the 35 V.P.I. selections that have been tested over a 14-year period, 8 have run consistently low in loose-smut infection.

The Influence of Tobacco-mosaic Virus on Cellular Oxidation. WOODS, MARK W., AND H. G. DUBUY. Three different oxidation components contributing to the rate of protoplasmic streaming in tobacco have been determined during progressive suffocation. The first of these, the "A component," ceases to function at a certain oxygen level, as measured by protoplasmic streaming. Later, a "B component" drops out, while, at the lowest oxygen tensions, protoplasmic streaming is energized only by a "C component." The A and B components can also be reversibly inhibited with NaCN or KCN. After infection with tobacco mosaic virus number 1, there is a reduction in the activity of the A component 48 to 62 hours after infection. The B and C components are not affected until the A component is completely inactivated, then there is a rapid *increase* in the activity of C. The B component is generally not affected. The 3 oxygen-sensitive components that energize protoplasmic streaming are believed to be respiratory catalysts, since measurements in the polarographic respirometer show a similar 3-step reduction in respiration rate during suffocation. The reversible inhibition of virus multiplication by NaCN, as previously reported, indicated a dependence of the virus mechanism on certain of these catalysts. The above data now indicate a specific action of the virus on a cyanide-sensitive respiration component.

Fungi Observed in the Trunks of Young Pine Trees in Natural and Artificial Established Forests. YORK, HARLAN H. This paper deals primarily with the results of cultural studies that the writer has made from the trunks of more than 500 white, red, Scotch, and jack pine trees in forest plantings, and of more than 100 white, red, and Virginia scrub pines that developed where the seed fell. The age of the trees ranged from 5 to 35 years. This work was done in connection with the writer's investigations of various root and root-crown diseases in the artificially established forests of the City of Rochester, near Springwater, New York. Summarized, the results are: 1. Observation of various species of fungi and bacteria were found in most of the nodes and internodes of examined trees, including the current season's growth. 2. The number and variety of organisms in apparently normal trees that developed where the seed fell were many fewer than in seemingly healthy individuals from the planted forests. 3. The trunks of trees showing very early symptoms of root and root-crown disturbances had many more and a greater variety of fungi than those showing no visible indications of disease. With the advance of disease in the parts below ground, there was a marked increase in the number and variety of these organisms in the trunk of the tree.

Relative Susceptibility of Young Pine Trees in Artificial and Natural Stands to Various Root, Root-crown, and Butt Fungus Parasites. YORK, HARLAN H. Investigations over a period of 12 years in forest plantings of white and red pines show that there is a definite relation between the way in which the roots of a tree are placed in the ground and root, root-crown, and butt diseases. Although the reactions to these parasites may be accentuated by abnormal biological and other factors in the soil and by the absence of proper silvicultural practices, the age of the tree and the way in which its roots are placed in the soil at time of transplanting undoubtedly play a very important part in influencing its susceptibility to fungus parasites that cause root, root-crown, and butt diseases.

The Effects of Copper Sprays on Tomato Yields. YOUNG, P. A. In experiments at Jacksonville, Texas, tomatoes were sprayed for the last 5 years to find copper spray materials cheaper and more beneficial than Bordeaux mixture. The last 3 seasons were rainy, resulting in serious damage by *Alternaria solani* and *Bacterium vesicatorium* in many local nonsprayed tomato fields. The best spray chemicals satisfactorily controlled tomato leaf and fruit diseases in that the sprays delayed the appearance and decreased the spread of parasites long enough to facilitate profitable yields of fruit. On the basis of 3-year averages, thousands of Marglobe plants sprayed with the following chemicals at recommended concentrations, gave the following percentages of the yields of the nonsprayed checks: Basicop 113 per cent, Bordeaux mixture 101 per cent; Coposil 106 per cent, Copper hydro "40" 110 per cent; Cuprocide 99 per cent, and Nichols Z–0 113 per cent. Yields ranged from 4.71 to 12.78 tons of marketable fruit per acre. The copper sprays only rarely caused necrotic spotting of the leaves.

The Inheritance of a Leaf Variegation in Beans. ZAUMEYER, W. J. Two types of leaf variegation resembling mosaic have recently been observed in beans, one of which appeared both on primary and trifoliate leaves and, the other, only on trifoliate leaves. The symptoms of the two are alike but are inherited differently. The inheritance of the first type is here reported. Results show that it is governed by at least 2 Mendelian recessive factors. In F_1 all plants from reciprocal crosses were green. In F_2 a ratio of 15 green to 1 variegated plant occurred. F_3 progenies from green F_2 plants segregated into 3 classes in the ratio of 7: 4: 4 as follows: (1) all green, (2) 15 green to 1 variegated, and (3) 3 green to 1 variegated, respectively. Many variegated recessive progenies and variegated parents did not breed true. It is believed, however, that they were homozygous for the 2 main factors for variegation. Modifying factors were probably responsible for the observed lack of true breeding. Out-crossing variegated plants with varieties not carrying the modifiers, produced good ratios in F_2 and F_3. Many variegated plants died and the survivors probably were carrying the modifiers. In the progeny of these plants, the character may again have been suppressed because of the modifiers, thus accounting for the high percentage of green plants in the recessive class.

Genetic Studies of Resistance to Six Physiologic Races of Bean Rust. ZAUMEYER, W. J. AND L. L. HARTER. The mode of inheritance in bean seedlings to 6 physiologic races of bean rust (1, 2, 6, 11, 12, and 17) was studied for 5 different crosses involving 6 bean varieties under greenhouse conditions. The range of resistance to susceptibility graded from 0 (immunity) to 10 (extreme susceptibility). Results proved that resistance to races 1 and 2 in the hybrids investigated was due to a single Mendelian factor. The results with hybrids inoculated with races 6, 11, 12, and 17 indicate that more than a single factor was involved. Resistance was dominant in the hybrids inoculated with races 1, 2, 6, and 12 and incompletely dominant in those inoculated with races 11 and 17. It is believed from the results with hybrids inoculated with races 11 and 12, that a major factor may govern resistance from grade 0 to 4 and a similar factor susceptibility from grade 5 to 10. Minor or modifying factors may be responsible for variable degrees of resistance and susceptibility found within major classes. Hybrids inoculated with race 11 showed a transgressive segregation, since one-fourth of the F_2 plants exhibited more resistance than the tolerant parent.

SOME PROBLEMS IN HANDLING AND INTERPRETING PLANT DISEASE DATA IN COMPLEX FACTORIAL DESIGNS[1]

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INTRODUCTION

Frequently, plant-disease data are expressed as percentages, or some relative scale of an index system is used. Bliss (3) and others (5, 6) have pointed out that when such data are to be subjected to the analysis of variance it may be found necessary to transform them to some new scale in order to construct a valid estimate of the generalized standard error. However, when percentage data are from experiments where large differences are secured for certain of the disease-control treatments, or where known checks are included, the resultant heterogeneity makes the problem of a reliable error term or of selecting the proper transformation more difficult. This is especially true where the data are derived from experiments of factorial design because interactions are of considerable interest;

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[1] Cooperative investigations between the Departments of Agronomy and Plant Pathology, Arkansas Agricultural Experiment Station, and the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

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[2] Grateful acknowledgment is made to W. G. Cochran for criticism of the manuscript and for many helpful suggestions.

yet, they may not always be interpreted with confidence on the transformed scale. In view of these difficulties plus the fact that the experimenter seldom has time to try out several types of analyses on a set of data, it is obvious that it would be helpful if there were some simple guide as to the need for transformations, as to the specific type to use for a given problem, and as to the nature of the changes that would be induced in the data. On the other hand, development of some simple but reliable generalized scheme for avoiding transformations might be found even more helpful. This note deals with some of these problems and is presented with the hope that it may be helpful to others who have encountered similar difficulty.

In the course of experimentation concerned with mineral nutrition of cotton in relation to its susceptibility to Fusarium wilt, the writers have obtained data that were suspected of needing transformation prior to the analysis of variance for the following reasons: First, the method of recording disease made each observation a percentage of the total possible disease within a plot. Second, the combined data were inclusive of considerable heterogeneity because of varietal specificity on the one hand and influence of imposed variations in nutrient supply on the other. Although each of these experiments differed in some respects from the others, as will be brought out in another report on these studies, all were inclusive of the same obstacles to the construction of a valid estimate of the generalized standard error. Therefore, the discussion will be limited to but one example, Experiment A.

EXPERIMENTAL EXAMPLE

This experiment was designed factorially to study N–P–K nutrition of 3 varieties of cotton in relation to susceptibility to Fusarium wilt in sand culture in the greenhouse. The 3 varieties were Cook 307 (considered wilt resistant), Rowden 2088 (intermediate or tolerant), and Half and Half (very susceptible). Twelve different nutrient solutions resulted from utilizing all possible combinations of 3 levels of nitrogen supply and 2 levels of phosphorus and potassium. Since each of the 3 varieties was treated with each of the 12 variations in nutrient supply, 4 replicate blocks gave a total of 144 plots. Each plot consisted of a 3-gal. earthenware jar containing 8 to 11 (usually 10) plants, all of which were inoculated[3] twice during the first 3 weeks of seedling growth. Unfortunately, from the standpoint of a theoretically ideal example, the pots in this experiment were arranged systematically instead of at random in each block (portion of the greenhouse bench). Fortunately, from the standpoint of appropriateness of applying analysis of variance to the data, there was little or no evidence for locational effect between benches or within benches with respect to development of wilt symptoms. In addition to the nonsignificant differences obtained between blocks, other tests, which may require no elaboration here, indicated that placement of the pots in this experiment was of little importance.

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[3] A description of this inoculation technique was presented in an earlier paper by the senior author (10).

The relative severity of the disease occurring in each plot was recorded as a code value representative of the percentage of the total possible disease severity. The first record of the diseased condition of the plants was made 3 days after the first definite symptoms of wilt were observed in the test. A similar record was taken each third day thereafter until termination of the test. Plants were recorded as being either healthy, slightly wilted, badly wilted, or dead. To each of these 4 severity classes the empirical values 0, 3, 7, and 10, respectively, were assigned. A disease code value for any one plot was formed by multiplying the number of plants recorded in each class by the respective severity values and dividing the sum of these products by the total possible disease values for that plot. This total possible would occur only when all plants within the plot were recorded as dead. For example, if the 10 plants in one plot were recorded as 3 healthy, 3 slightly wilted, 1 badly wilted, and 3 dead, the disease code value for that plot would be $(3 \times 0) + (3 \times 3) + (1 \times 7) + (3 \times 10) / (10 \times 10) = 0.46$ or 46 per cent of the possible. In Experiment A there were 9 such disease records obtained for each of the 144 plots. An accumulated disease-code value was formed by averaging the 9 code values obtained from the individual recordings. Each of these final disease-code values is a mean percentage with its magnitude dependent on the number of plants affected, the earliness of the appearance of disease symptoms in each of these plants, and the relative severity of the damage to the plant caused by the wilt pathogen. These final, or accumulated, code values of disease for each of the 144 plots of Experiment A are assembled in table 1.

A study of the mean values given in table 2 indicates that no analysis of variance is needed to establish the significance of the susceptibility of Half and Half in comparison with Cook or Rowden, that increase of N or P induces greater susceptibility, and that the addition of K results in a reduction of the amount of wilt. If one desired information on main effects only, the value of a statistical analysis of the data might be open to question and, although a theoretical need for transformations might be shown to exist, the practical problem of suitable error terms would not arise. However, based on the inconsistency of replicate behavior shown in table 1, there are, as between Cook and Rowden, one or two comparisons that are open to question. As to the mean values for the first-order interactions given in table 2, it would be difficult to arrive at a satisfactory estimation of the significance of these, after making a net allowance for main effects, without statistical treatment. However important the main effects in a factorial experiment may be it is not infrequent that the experimenter has as much or possibly more interest in the information that can be secured on the extent to which levels of one factor may modify the influence of another, and, as in the experiment under consideration, it would be inefficient procedure to confine a study of the data to main effects, or to fail to make an appropriate statistical analysis—one that would give an unbiased interpretation for interaction, as well as for main effects.

TABLE 1.—*Disease code values in the 144 plots of experiment A*

	Coded values of disease in each plot (jar)												Sums
Level of N	Low				Medium				High				
" " P	Low		High		Low		High		Low		High		
" " K	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	
Cook 307													
Block 1	6.0	0	0.9	0	0	0	1.9	0	7.2	0	26.2	0	42.2
" 2	2.1	0	0	6.5	0	0	4.1	0	0	0	10.8	1.5	25.0
" 3	0	0	0	0	0	8.0	5.6	0.8	7.2	0	2.2	0	23.8
" 4	0	0	3.0	0	0	1.5	1.4	0.4	0	0	7.9	2.4	16.6
Rowden 2088													
Block 1	1.1	9.1	0	0	7.9	0	14.4	4.6	11.3	2.6	5.2	13.0	69.2
" 2	0	0	3.0	2.2	0	0	4.4	0	1.2	2.2	15.9	9.4	38.3
" 3	4.1	6.1	12.0	0	0	6.7	8.4	0.4	0	3.3	16.0	14.4	71.4
" 4	2.5	0	0	2.2	0	0	5.0	5.0	7.4	3.0	11.4	12.7	49.2
Half and Half													
Block 1	9.5	24.6	25.7	25.5	9.6	23.4	31.1	19.9	40.2	8.2	47.7	42.7	308.1
" 2	3.7	3.2	12.2	8.3	7.9	16.4	34.0	34.7	6.7	0	78.1	22.1	228.3
" 3	8.0	0	8.2	33.6	7.5	3.5	32.6	11.6	42.3	22.2	55.5	37.1	262.1
" 4	5.0	0	33.8	9.9	24.5	1.9	51.1	31.9	9.7	38.1	39.2	10.1	255.2
Sums	42.0	43.0	98.8	88.2	57.4	61.4	194.0	109.3	133.2	79.6	316.1	165.4	1,388.4

TABLE 2.—*Means of disease code values for all main effects and first-order interactions*

Variety	N levels			P levels		K levels		Mean for variety
	Low	Med.	High	Low	High	Low	High	
Cook	1.16	1.48	4.09	1.35	3.15	3.60	0.88	2.24
Rowden	2.64	3.55	8.06	2.85	6.65	5.47	4.04	4.75
Half and Half ...	13.20	21.35	31.24	13.17	30.69	25.99	17.87	21.93
Mean	5.67	8.79	14.46	5.79	13.50	11.69	7.60	....

N levels	P levels		K levels		Mean for N
	Low	High	Low	High	
Low	3.54	7.79	5.87	5.47	5.67
Med. ...	4.95	12.64	10.48	7.11	8.79
High ...	8.87	20.06	18.72	10.20	14.46
Mean ...	5.79	13.50	11.69	7.60	....

P levels	K levels		Mean for K
	Low	High	
Low	6.46	5.11	5.79
High ...	16.91	10.08	13.50
	....	....	....
	11.69	7.60	....

The authors were unable to find any satisfactory general rule of procedure for the analysis of plant-disease data of this type derived from complex factorial experiments, and felt it desirable to make a study of several methods for handling such data before proceeding blindly with the analysis of all of the data on hand.

TRANSFORMATIONS IN COMPLEX FACTORIAL EXPERIMENTS

Bliss (2, 3), Bartlett (1), Cochran (6), and others (5, 7, 11) have published articles concerned with the use of transformations in the analysis of variance. They have pointed out that data expressed as percentages may often need transformation prior to analysis. Transformations also are cited as being useful in the analysis for the purpose of equalizing the variance of quantities of widely different magnitudes that it is desired to include in the same analysis, serving to reduce the observations to a scale on which the effects of associated variables are more nearly constant.

Three types of transformation that have been most widely utilized were studied: the angular (or inverse sine), the square-root, and the logarithmic. Each of these offers a somewhat different type of correction and each places the data on a slightly different scale.

The angular transformation has been recommended for binomial distributions where the observations are expressed as fractions or percentages, when treatments are not equally effective and when block variability is large. It is also recommended for use where the original data are discrete—being based on a determinate number of trials or cases. In the transformation, each percentage, or estimate of p, is considered as the value of $\sin^2 \theta$ and the actual analysis is performed on the corresponding values of θ.[4]

The square-root transformation is recommended for those cases where a

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[4] The original article by Bliss (2) contains a table that can be used in making the angular transformation. The text by Fisher and Yates (7) contains tables that can be used for making all three transformations.

Poisson distribution is encountered and where the variance is proportional to the mean. It is performed by replacing each original observation with its square root.[5] If many of the original observations are less than 10, then a better correction might be offered if some constant value is added to each observation before extracting its square root.

The logarithmic transformation has been suggested for data wherein the effects of treatments are proportional to the base levels from which they are measured, or where the standard errors are proportional to the means. The relationship utilized is $y = \log x$,[6] the observations being replaced by their natural logarithms. When zero values and small numbers predominate in the data, then the addition of a small constant value to each observation before taking its logarithm will give better equalization of the variances.

Figure 1 presents the distribution of the errors of the data of table 1 within variety classifications after allowance for nutritional effects. The distributions of errors for Cook and Rowden varieties are evidently normal and homogeneous. The errors for Half and Half variety appear to have a skew distribution and to be nonhomogeneous with the errors for the other two varieties. The values for the standard deviations of the 3 distributions are also given in figure 1. From these it is evident that the observations within varieties cannot be expected to have the same variance, and that the pooling of variety variances in the same analysis might be questionable.

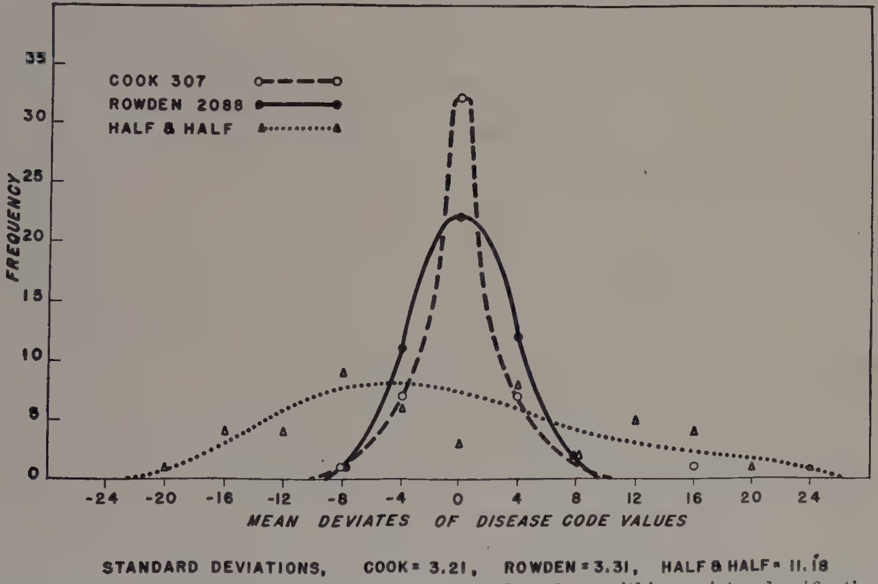

FIG. 1. Distribution of errors for disease code values within variety classifications after allowance for nutritional effects.

When these data were stratified for distribution of errors within the respective nutritional contributors, after making an allowance for the effects of all other factors, except the one being studied, there was little evidence of the heterogeneity that characterized the variety distributions of figure 1. There are, however, a few observations in these data that fall considerably outside the expected frequency for a given category of effects. For example, the observations for Cook variety in the high N-high P-low K treatment in Block I has a value of 26.2, which not only falls entirely outside the distribution of the other values in the Cook populations, but is almost 4 times as large as the mean of the other 3 replicates for this treatment-variety combination (Table 1). Also, the observation of 0 disease in the susceptible Half and Half variety in the high N-low P-high K treatment of Block II is very anomalous in view of the fact that the mean disease index for the other 3 replicates in the group is 26.5. A few other discrepancies could be pointed out for these data, but the above will suffice. It is suggested that for a precise estimation of the biological significance of such data, values of dubious validity, traceable to a certain degree to extraneous causes, may be replaced by values derived by a missing-plot technique such as described by Yates (12). The writers gave serious consideration to substituting missing values for some of these widely divergent results, but, in the end, were unable to decide where to draw the line without being "too arbitrary," plus the fact that, although the pots had been observed daily, we could do little more than speculate as to causes of failure for the results to be duplicated more closely; hence the feeling that it would be difficult to justify throwing out results that did not jibe.

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[5] See footnote 4.

[6] See footnote 4.

The selection of the correct transformation that will apply to data such as these is complicated because any given transformation may not fully ameliorate all of the difficulties encountered. That is, the unequal effects of treatment and varieties and any bias due to analysis of percentages might be corrected by the use of the angular transformation. Since the standard errors and the variances are roughly proportional to the mean,[7] the logarithmic and square-root transformations might merit consideration. However, since the variances increase more rapidly than the means, whereas the standard errors increase more slowly than the means, something between the square-root and the log transformations appears to be needed. The selection of the transformation might be facilitated if these data were subjected to the analysis of variance following the use of each of the 3 suggested transformations. That is, an examination of the results of the analyses might disclose which of the three has placed the data on a new scale that is devoid of the greatest number of sources of bias included in the original data. Inasmuch as the original data contained so many zero observations,

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[7] Please refer to table 5, which shows both effect and error variance for each of the three varieties. These are reproduced below, together with the standard errors and mean effects for each variety.

Variety	*Variance*		*Standard error*	*Mean*
	Effect	*Error*		
Cook	954.00	459.53	21.437	2.24
Rowden	1,206.12	450.00	21.213	4.75
Half & Half	14,217.24	5,594.78	74.805	21.93

it has seemed advisable to make the transformation to the logarithmic and square-root scales after the addition of a small constant value (+2) to each original observation. Since the logarithmic transformation cannot be used directly for zero values, the addition of a small constant not only becomes rather necessary but also provides a means of transformation that is intermediate in effect between these direct logarithmic and square-root conversions. The analysis of variance of the data on the original scale, and following transformation to each of the 3 new scales—the angular, the square-root, and the logarithmic—are shown in table 3.

The results of these analyses may now be examined to see if any or all of the transformations have facilitated the interpretations of the results, and also if the generalized standard error can be considered a valid estimate for testing all variances on any of the new or transformed scales. A thorough examination of the 4 analyses shown in table 3 will disclose that except for K differences, significance for main contributors, as shown by F values, has been strengthened through all transformations, viz., the F value for variety differences (V) was found to be 19.32 in the analysis of the original data, while the F values for the (V) differences in the analyses of transformed data were 30.21, 29.28, and 31.93—for the angular, square-root, and logarithmic data, respectively. However, each transformation has so conditioned the data that many of the interactions that were found significant when measured on the original scale are found to be without significance when measured on the transformed scale. When an interaction does become significant on a transformed scale, full cognizance must be given to the fact that a new criterion of independence is set up by the transformation. For example, on the log scale the mean response for any given combination of factors is a *geometric mean,* and comparisons between these means are made on a proportionate basis. Hence, if high phosphorus supply induces twice as much disease susceptibility as low phosphorus supply within the low potassium level, and also twice as much within the high potassium level, then these factors are acting independently on the log scale, although the arithmetical differences in the untransformed data might have been of sufficient disparity to cause the interaction to have appeared significant. Although the logarithmic conversion does set up a definition of independence that may be very logically used in studying the interactions in certain data, the angular and square-root transformations induce relationships within the data that are not easily interpreted when one wishes to study interactive effects.

In considering the interpretation of logarithmic comparisons it is to be noted that the conversion as employed by Williams (11) and others is usually carried out in the form $y = \log x$ or, in case small numbers and zeros predominate, the form $y = \log (x+1)$ is preferred. Since no examples have been found in the literature where the larger constant (+2), which was used here to establish positive values for the zero observations, questions may be raised as to the effect of the larger constant on the range of means in this

TABLE 3.—*Analyses of variance*[a] *of the data from experiment A in the original scale and following transformation to angular, square-root, and logarithmic scales*

Source of variation	Degrees of freedom	Analyses conducted on data				
		As original disease code values	following transformation to			
			Angles ($p = \mathrm{Sin}^2\,\theta$)	Square roots ($y = \sqrt{x+2}$)	Logs ($y = \log x + 2$)	Logs ($y = x + 1$)
		Mean square	Mean square	Mean square	Mean square	Mean square
V	2	5,512.84**	5,256.76**	90.9270**	36.4035**	53.6333**
N	2	954.72**	869.77**	14,7240**	5.6544**	8.7316**
V × N	4	263.87**	111.94	2.0780	0.4535**	0.8297
P	1	2,140.60**	2,325.65**	41.5058**	13.5057**	19.8470**
V × P	2	877.82**	352.95**	8.4956	1.4215*	1.4208
N × P	2	144.74	116.03	1.3141	0.7500	1.0723
V × N × P	4	11.42	32.01	1.0122	0.5275	0.8506
K	1	602.70**	791.95**	7.6868*	4.8870**	8.3424**
V × K	2	151.14	86.26	2.6081	0.2956	0.4499
N × K	2	202.22*	96.43	2.5245	0.4587	0.6370
V × N × K	4	83.43	68.42	0.2899	0.5264	0.7610
P × K	1	270.60*	191.14	1.7680	0.6551	0.7368
V × P × K	2	62.36	4.72	0.4843	0.2351	0.5433
N × P × K	2	46.27	78.05	1.2566	0.7259	1.4627
V × N × P × K	4	42.60	30.49	0.2804	0.3137	0.5616
Blocks	3	85.36	124.61	3.4098*	0.8731	1.1419
Error	105	63.08	59.75	1.1766	0.4021	0.6539
X^2 test for heterogeneity of variety variances	....	$X^2 = 61.8249$**	$X^2 = 10.0977$**	$X^2 = 10.74735$**	$X^2 = 2.01318$	....

Analysis carried out following Snedecor's (8) machine method for multiple degree of freedom comparisons. Significance derived from Snedecor's table of F values:

* Significant, odds of 19 to 1, 5 per cent level.

** Highly significant, odds of 99 to 1, 1 per cent level.

experiment. Consequently, there has been added a column in table 3 to show the analysis with $y = \log(x + 1)$. It will be seen that the principal difference between the two log comparisons is that no interactions were found to be significant at even the lower odds of 19:1 when $\log(x + 1)$ was used, whereas, with $\log(x + 2)$, interaction V × P reached significance. For reasons mentioned in the foregoing, it was felt that the larger constant (+2) would be preferable. Hence, unless otherwise stated, the discussions relative to transformation refer to the use of +2.

A determination of the validity of the generalized standard error can be made in several ways. In making similar measurements, Clark and Leonard (5) have employed a modified X^2 test, developed by Stevens (9). They have found it valuable for testing the assumption that each of a set of variances estimates a single variance formed when they are pooled and averaged; taking into account only those degrees of freedom associated with the variances to be tested. This modified X^2 test has been utilized here to test the homogeneity of the variances between the three varieties, since the variety factor seems to offer the greatest observed source of variation in the experimental data. This test was applied to the data prior to transformation and to each set of data following the 3 different transformations. These obtained X^2 values were tested for significance by reference to a table (8) of X^2 values for 2 (3–1) degrees of freedom and each of these four obtained values has been entered under the appropriate analysis in table 3.

This test shows that each of the transformations has reduced the heterogeneity associated with variety variances in the original scale, but, according to this test, only the logarithmic transformation has reduced this heterogeneity below the 1 per cent level of expectancy, since X^2 value for this latter transformation was only 2.0139 whereas the value of 9.210 is required for significance at the 1 per cent level. Thus, of the 3 transformations used, only the logarithmic—utilizing the form $y = \text{logs}\ (x + 2)$—could be validly used for testing all main effects by a generalized standard error. Futhermore, the definition of independence as set up by even the logarithmic transformation may not be the most suitable for the interpretation of the data at hand. It is evident that if interpretations were made solely from the analysis of the log data (Table 3), the only interaction to attain significance would be V × P. On this basis it is indicated that disease development, as influenced by level of phosphorus supply, is disproportionate among the different varieties, whereas the lack of significance for other interactions indicates that composite main effects are independent as far as the proportionality of their relationships is concerned. Furthermore, when the analysis is carried out, utilizing the form $y = \log(x + 1)$, it could be inferred that all main effects were acting independently.

ANALYSIS OF THE DATA BY SINGLE DEGREES OF FREEDOM

The single degree of freedom analysis, as explained by Brandt (4) and Snedecor (8), together with the use of specific error terms for testing each degree of freedom has been suggested as a means for satisfactorily evaluating the significance of comparison in heterogeneous material where confidence cannot be placed in the pooled error. Thus this method of analysis may be considered as a means of avoiding the necessity for transformations. Some objection has been raised to this proposal due to the fact that when each effect is tested by its own block interactions there are so few degrees of freedom from error that the variance ratio required for significance is very large in comparison with the variance ratio required in the fully pooled analysis. The writers have had considerable experience with the single degree of freedom type of analysis for plant-disease data and are convinced that it is a very important development for handling most of the data with which they have had to deal, and do not feel that the objection cited above is of great consequence provided there are from 5 to 8 degrees of freedom for interaction by blocks with any treatment, or possibly fewer if block variability is relatively negligible. In complex factorial experiments, where there are rarely more than 3 to 4 true blocks and often only 2, the above objection has been found to be a very real one frequently resulting in finding "high significance" where it is apparent that none could exist, or failing to have significance indicated when differences are obviously real. In such cases it has been found that what appears to be a much more reliable interpretation may be had by partitioning the pooled error to form groups that are larger than the specific errors. Perhaps "partitioning of error" may be used to distinguish, in the discussion that follows, these methods of grouping error comparisons from "specific error terms" (all block interactions with a treatment comparison) and the "fully pooled error." The specific-error advocates may argue that the larger grouping obtained by what is termed above as partitioning of error would tend to give an overestimate because of a piling up of degrees of freedom in portions of error to be used and the advocates of the pooled error may still regard with suspicion the small, in comparison with the total pooled error, number of degrees of freedom in this partitioned error and feel that this would tend to give an underestimate. The experience the writers have had to date indicates that the use of the partitioned error, which is intermediate between the specific error term and the fully pooled error, increases the reliability of the estimate in complex designs and may provide a safe procedure for avoiding transformations for much of the data with which the plant-disease investigator has to deal.

The present evidence indicates that there may be considerable latitude as to choice for grouping of error terms, provided one keeps in mind the sources for upsets in the generalized error. The writers have not had opportunity to explore the dangers of unwise grouping or to formulate any general rules as to what may constitute wise or unwise groupings, except to suggest that the process does not seem to be so delicate but that the experimenter may rely on his own good judgment. As will be pointed out in the following discussion, the present example is not so striking for this particular purpose as might have been anticipated or as has been encountered, but it is believed that it may serve very well to illustrate the problem.

TABLE 4.—*Analysis of variance of disease code values from experiment A by single degrees of freedom. Showing (A) significance tested by fully pooled error—105 D/F, and (B) significance of each effect tested on its specific error term (3 degrees of freedom)*

Source of variation	Mean square		A	B
	Effect	Error		
V_1[a]	152.15	8.39	......	*
V_2	10,874.44	30.97	**	**
N_1	1,857.68	12.91	**	**
N_2	51.76	72.53	......	......
$V_1 \times N_1$	24.75	11.00	......	......
$V_1 \times N_2$	2.34	31.00	......	......
$V_2 \times N_1$	1,025.83	87.84	**	*
$V_2 \times N_2$	2.56	212.68	......	......
P	2,140.60	58.14	**	**
$V_1 \times P$	23.50	16.29	......	......
$V_2 \times P$	1,732.15	36.52	**	**
$N_1 \times P$	289.47	117.66	*	......
$N_2 \times P$	0.01	31.82	......	......
$V_1 \times N_1 \times P$	20.48	16.77	......	......
$V_1 \times N_2 \times P$	2.34	17.48	......	......
$V_2 \times N_1 \times P$	1.88	197.76	......	......
$V_2 \times N_2 \times P$	21.00	58.67	......	......
K	602.70	2.21	**	**
$V_1 \times K$	10.07	11.00	......	......
$V_2 \times K$	292.22	53.19	*	......
$N_1 \times K$	394.88	103.12	*	......
$N_2 \times K$	9.57	36.39	......	......
$V_1 \times N_1 \times K$	35.11	5.72	......	......
$V_1 \times N_2 \times K$	46.41	28.84	......	......
$V_2 \times N_1 \times K$	249.80	133.04	*	......
$V_2 \times N_2 \times K$	2.40	128.45	......	......
$P \times K$	270.60	26.00	*	*
$V_1 \times P \times K$	1.06	23.09	......	......
$V_2 \times P \times K$	123.64	135.54	......	......
$N_1 \times P \times K$	76.15	95.61	......	......
$N_2 \times P \times K$	16.39	100.54	......	......
$V_1 \times N_1 \times P \times K$	70.98	28.77	......	......
$V_1 \times N_2 \times P \times K$	0.44	7.82	......	......
$V_2 \times N_1 \times P \times K$	96.62	129.89	......	......
$V_2 \times N_2 \times P \times K$	2.40	140.19	......	......
Blocks (3 D/F)	85.36	....	......	......
Error	....	63.08 (105 D/F)		......

[a] The single degree of freedom comparisons are made as follows:
V_1 = difference between Cook and Rowden.
V_2 = difference between Half & Half and the mean of Cook and Rowden.
N_1 = difference between low and high levels.
N_2 = difference between medium level and mean of low and high levels.
P = difference between low and high levels.
K = difference between low and high levels.

Reference to tables 4 and 6 shows comparisons of the above described methods of treating error derived from the nontransformed code values. Table 4 gives an indication of the source of variability in error and, in addition to the tests discussed earlier, which showed the need for transformations, shows clearly why the fully pooled error is unsatisfactory in this case, *i.e.*, the greatest mean square is shown to be that for the V_2 comparison (difference between Half and Half, and the mean of Cook and Rowden), with an error term comparably large; and while the mean square for K differences is much smaller, it is associated with a comparably small specific error term. Cochran (6) has shown that when a condition of this kind is encountered, the comparisons can be restratified to form several error terms, each of which is practically exclusive of errors to which the variances within the group are not subject. Perhaps the most logical basis for the regrouping should be that of treatments. The modified X^2 test, as used by Clark and Leonard (5), offers a means of testing for the major sources of heterogeneity in order to apprehend a basis for this regrouping. This test was applied to each major source of variation within the experiment (viz., V,N,P, and K) and the resultant X^2 values are given in table 5, which shows also the method of applying this test to the variety variances. Obviously, the relative consistency of P and K differences should not be subjected to testing by an error term inclusive of the heterogeneity associated with variety, or, perhaps, even with nitrogen, differences. To accomplish such a stratification of error terms, it was deemed advisable to form several homologous groups with P, K, and P × K forming the first group, error (a). Then all subsequent groups result from combining this group with each of the 8 other sources of variation: V_1, V_2, N_1, N_2, $V_1 \times N_1$, $V_1 \times N_2$, $V_2 \times N_1$

TABLE 5.—*Heterogeneity test applied to the original data from experiment A. The calculation is carried out on the variety variances and only the resultant X^2 values are shown for the comparisons of variances due to nitrogen, phosphorus and potassium*

Source of variation	D/F	Analyses of data within		
		Cook	Rowden	Half and Half
Total	47	954.00	1,206.12	14,217.24
Treatments	11	464.98	692.19	8,341.63
Blocks	3	29.48	63.95	280.83
Variance	33	459.53	450.00	5,594.78

For varieties

$$X^2 = \frac{(V_r - V)^2\,C + (V_r - V)^2\,R + (V_r - V)^2\,H(n_r - 1)}{2V^2}$$

$$X^2 = \frac{[(459.53 - 2{,}168.10)^2 + (450.00 - 2{,}168.10)^2 + (5{,}594.78 - 2{,}168.10)^2]\ (33)}{2(2{,}168.10)^2}$$

$$X = \frac{581{,}236{,}090.71}{9{,}401{,}315.22} = 61.8249$$

For varieties, $X^2 = 61.8249^{**}$ } 9.210 required for high significance.
For N levels, $X^2 = 13.1510^{**}$ }
For P levels, $X^2 = 0.6273$
For K levels, $X^2 = 0.6273$

In this test, $X^2 = \frac{S(V_r - V)^2\,(n_r - 1)}{2V^2}$
where n_r represents the number of variates within each level of the factor under consideration diminished by the number of degrees of freedom due to other known contributors whose variance is initially removed; V_r represents the variance within each level of the factor diminished by variance due to known contributors; and V represents the mean of the V_r variances.

and $V_2 \times N_2$ to the formation of 9 separate error terms as shown at the left in table 6. By reference to table 6 it may seem that effects with relatively large individual errors are estimated more leniently by the regrouped error, and *vice versa.* It is believed that, although block differences in this case were negligible, 3 degrees of freedom for specific error were too close to the minimum to be regarded without suspicion, and that the regrouped errors (or partitioned errors) have resulted in a more reliable estimate.

It has been pointed out by aid of figure 1 and table 5 that the main source of heterogeneity in the data under discussion is found in the disparity in error variances for observations within the variety Half & Half as distinguished from those variances for the varieties Cook and Rowden. Comparisons between the latter two varieties, designated by V_1, have definitely smaller individual errors than those involving the variety Half & Half (V_2 comparisons and pooled variety effects). In table 6, method C has 9 groups of error terms, the first of which is formed by P and K (and $P \times K$), which had nonsignificant X^2 values in table 5. Each succeeding group is formed by the addition of an N or V comparison, both of which had highly significant X^2 values. Method D has P, K and $P \times K$ plus V_1 (including interactions with N) in one group, N, P, $N \times P$ and $P - K - V_2$ interactions in the second, and $V_2 - N$ interactions in the third group. Method E has only 2 groups, 1 of which includes all V_1 comparisons; the other includes the remaining comparisons. By comparing the 3 columns of asterisks in table 6, it is evident that there is but little discrepancy due to the use of the 3 methods for grouping of error terms. Two rather important differences result from the grouping of errors, the changes from low significance for V_1, method B, given in table 4, to high significance with methods C, D, and E. This is in accord with all the other types of analyses made, with the exception that the fully pooled error from nontransformed data fails to show significance for V_1. Also, $N_1 \times K$ reaches low significance. From table 8 it may be seen that this coincides with the analysis on the proportionate basis. In comparing methods C and D it is noted that $P \times K$ changes from low to high and $N_1 \times P$ and $V_2 \times K$ attain low significance in method D. In going from method D to E it is seen that $P \times K$ lost significance (a fact that does not agree with any other analysis, except where transformations were used), $N_1 \times P$ is lost, $V_1 \times N_1 \times P \times K$ reaches low, and $V_2 \times N_1$ changes from low to high.

While in this greenhouse experiment, where there was little block effect, method B with only 3 D/F in the specific errors gave a surprisingly good interpretation of the algebraic differences for comparisons despite the lack of homogeneity in the coded values; it, nevertheless, should be pointed out that method B appears to be inferior to methods C and D. This is brought out in the preceding paragraph and can be further verified by comparing the results for other methods of analysis shown in tables 3, 4, 6 and 8. Method C appears to have a sufficient number of degrees of freedom to be fairly reliable, but method D gives some additional information that is substantiated by other tests, as well as by observational interpretation of the results. Method E appears to have no particular merit, except that if it had been decided upon in advance, the larger error (69 D/F) could have been obtained by subtraction. It may not be a particularly logical grouping, but the purpose of including it is to show that no great discrepancy in interpretation has been caused. It would seem, from the preceding and

TABLE 6.—*Regrouping variances on the basis of homogeneity and similar treatments into C, nine groups; D, three groups; and E, two groups. Data from experiment A, analyses as original code values*

Individual comparisons grouped by like treatments				Significance[a] as tested by method		
Source of variation	D/F	Mean Squares				
		Effect	Error[b]	C[c]	D[d]	E[e]
P	1	2,140.60	58.14	**	**	**
K	1	602.70	2.21	**	**	**
$P \times K$	1	270.60	26.00	*	**	
Error (a)	9		28.78			
N_1	1	1,857.68	12.91	**	**	**
$N_1 \times P$	1	289.47	117.66		*	
$N_1 \times K$	1	394.88	103.12	*	*	*
$N_1 \times P \times K$	1	76.15	95.61			
Error (b)	12		82.32			
N_2	1	51.76	72.53			
$N_2 \times P$	1	0.01	31.82			
$N_2 \times K$	1	9.57	36.39			
$N_2 \times P \times K$	1	16.39	100.54			
Error (c)	12		60.32			
V_1	1	151.25	8.39	**	**	**
$V_1 \times P$	1	23.50	16.29			
$V_1 \times K$	1	10.07	11.00			
$V_1 \times P \times K$	1	1.06	23.09			
Error (d)	12		14.69			
V_2	1	10,874.44	30.97	**	**	**
$V_2 \times P$	1	1,732.15	36.53	**	**	**
$V_2 \times K$	1	292.22	53.19		*	
$V_2 \times P \times K$	1	123.64	135.54			
Error (e)	12		64.06			
$V_1 \times N_1$	1	24.75	11.00			
$V_1 \times N_1 \times P$	1	20.48	16.77			
$V_1 \times N_1 \times K$	1	35.11	5.72			
$V_1 \times N_1 \times P \times K$	1	70.96	28.77			*
Error (f)	12		15.56			
$V_1 \times N_2$	1	2.34	31.00			
$V_1 \times N_2 \times P$	1	2.34	17.48			
$V_1 \times N_2 \times K$	1	46.41	28.84			
$V_1 \times N_2 \times P \times K$	1	0.44	8.15			
Error (g)	12		21.37			

TABLE 6.—*Concluded*

Individual comparisons grouped by like treatments				Significance[a] as tested by method		
Source of variation	D/F	Mean Squares				
		Effect	Error[b]	C[c]	D[d]	E[e]
$V_2 \times N_1$	1	1,025.83	87.84	*	*	**
$V_2 \times N_1 \times P$	1	1.88	197.76			
$V_2 \times N_1 \times K$	1	249.80	133.04			
$V_2 \times N_1 \times P \times K$	1	96.62	129.89			
Error (h)	12		137.13			
$V_2 \times N_2$	1	2.56	212.69			
$V_2 \times N_2 \times P$	1	21.00	58.67			
$V_2 \times N_2 \times K$	1	2.40	128.45			
$V_2 \times N_2 \times P \times K$	1	2.40	140.19			
Error (i)	12		135.00			
Blocks	3	85.36				
(Blocks tested by fully pooled error (63.08)—105 D/F.)						

[a] Asterisk (*) signifies odds of 19:1; double asterisk (**), odds of 99:1.

[b] There are 3 degrees of freedom in error for each individual comparison for effect.

[c] In method C, errors are grouped into 9 terms as shown; *i.e,* error (a), error (b), etc.

[d] In method D, errors are grouped into 3 terms; error I, 45 D/F, with a mean square of 20.10 formed from errors (a), (d), (f), and (g) of method C; error II, 36 D/F, mean square of 68.90 from errors (b), (c), and (e) of method C; error III, 24 D/F, with a mean square of 136.07, from errors (h) and (i) of method C.

[e] In method E, errors are grouped into 2 terms only: Error I, 36 D/F, with a mean square of 17.21 from errors (d), (f), and (g) of method C; error II, 69 D/F, mean square of 86.60 formed from the remaining 6 terms of method C.

from similar analyses that have been made to date of factorial experiments that include some quite large differences for treatment, even though the data are recorded as percentages, that the problem of an unsatisfactory, fully pooled error and, hence, the need for using transformations, may be very easily and safely avoided by partitioning the error term. It is suggested that some method of partitioning similar to method C or D merits consideration, although simpler methods of removing the principal causes of disturbance from the pooled error may render the remainder reasonably satisfactory for testing most of the comparisons in a complex experiment.

TREATMENT OF DATA TO ESTABLISH A PREFERRED INTERPRETATION OF INTERACTIONS

It has already been pointed out by Cochran (6) that the definition of independence of related factors, expressed by the usual test for interactions, may not be the one that will give the desired interpretation of the results. In this particular experiment it was felt that independence should be considered as indicative of disproportionate responses, instead of indicating actual algebraic differences in response—as is the case in the usual test for interaction. For example, an increase in the supply of a certain nutrient might cause an increase in disease in a resistant variety from 4 per cent to 8 per cent and an increase in a susceptible variety from 40 per cent to 80 per cent. The straightforward analysis of variance might show these differences in increase (4 per cent and 40 per cent) to be a significant interaction; whereas, in relation to disease potentialities of the two varieties, these differences are merely proportionate, since each shows the same percentage increase (100 per cent). As shown by the analyses in table 6, the varietal responses to nutrient effects are quite distinct, yet the magnitude of the variances in Half and Half would tend to obscure those for the other two varieties in a pooled variety analysis.

Fortunately, it is easy to transform the original data so that independence of any treatment effect can be measured against base levels for varieties, etc. This has been done for the original data of Experiment *A* by dividing each observation within a variety by the total of all observations for that variety. Each new value then becomes a percentage of the total observed disease within each variety and the sums for each variety become 100. Table 7 presents the summaries for the various main effects and first-order interactions after proportionate treatment with varieties as base levels. Thus, with the marked disparity in varietal response removed, it can be seen by inspection whether or not there is actually any difference in the proportion of nutrient response in different varieties (nutrient × variety interactions) since, following this treatment, differences in proportionality will be revealed as actual differences in summation—or in mean-responses. For example, in table 7, it is quite obvious that there is no difference in proportionate response to increase in level of P supply in the 3 varieties, since the

TABLE 7.—*Summary tables for all first order interactions of the data after each observation has been made proportionate to respective varietal means*

	Σ Cook	Σ Rowden	Σ H. and H.	Mean
ΣN_1	17.20	18.53	20.03	18.58
ΣN_2	22.01	24.89	32.44	26.44
ΣN_3	60.77	56.54	47.49	54.93
ΣP_1	29.73	30.02	30.00	29.91
ΣP_2	70.25	69.94	69.96	70.05
ΣK_1	80.39	57.50	59.24	65.71
ΣK_2	19.59	42.46	40.72	34.25

	ΣN_1	ΣN_2	ΣN_3	Mean
ΣP_1	22.67	24.21	42.87	29.91
ΣP_2	33.09	55.13	121.93	70.05
ΣK_1	31.18	48.48	117.47	65.71
ΣK_2	24.58	30.86	47.33	34.25

	ΣP_1	ΣP_2	Mean
ΣK_1	53.05	144.08	98.56
ΣK_2	36.70	66.07	51.38
Mean	44.87	105.07	

actual differences shown (after proportionate treatment) are nearly identical—these being 40.52, 39.92, and 39.96 for Cook, Rowden, and Half and Half, respectively. It would have been difficult to arrive at this conclusion by an examination of mean responses in the original data (Table 2). Here the differences attributable to increase in level of P supply are quite different, and it would be difficult to decide if they were or were not disproportionate—actual differences being 1.80, 3.80, and 17.52 for Cook, Rowden, and Half and Half, respectively.

The analysis of the data as proportions within the respective varieties is shown in table 8. Since the sums for each variety are 100 per cent, the mean square for variety differences must be zero. The differences due to nutritional treatment within each variety, however, are all measured within

TABLE 8.—*Analysis of variance of data from experiment A after the disease code values have been recalculated as percentages of total within each variety. Analysis by single degrees of freedom. All effects tested by fully pooled error*

Source of variation	Mean square found for effect
V_1	0.0000
V_2	0.0000
N_1	123.8512**
N_2	13.2956
$V_1 \times N_1$	0.4830
$V_1 \times N_2$	0.3906
$V_2 \times N_1$	3.7018
$V_2 \times N_2$	5.0550
P	100.6677**
$V_1 \times P$	0.0037
$V_2 \times P$	0.0009
$N_1 \times P$	49.0776**
$N_2 \times P$	2.6526
$V_1 \times N_1 \times P$	0.0264
$V_1 \times N_2 \times P$	2.9403
$V_2 \times N_1 \times P$	13.6107
$V_2 \times N_2 \times P$	2.4937
K	61.8320**
$V_1 \times K$	21.8120*
$V_2 \times K$	5.2272
$N_1 \times K$	42.0555**
$N_2 \times K$	5.9800
$V_1 \times N_1 \times K$	33.5820*
$V_1 \times N_2 \times K$	25.5646*
$V_2 \times N_1 \times K$	3.0754
$V_2 \times N_2 \times K$	1.8814
$P \times K$	26.4024*
$V_1 \times P \times K$	5.8905
$V_2 \times P \times K$	2.1805
$N_1 \times P \times K$	10.3359
$N_2 \times P \times K$	5.1574
$V_1 \times N_1 \times P \times K$	37.4238**
$V_1 \times N_2 \times P \times K$	0.3300
$V_2 \times N_1 \times P \times K$	0.5676
$V_2 \times N_2 \times P \times K$	2.1928
Blocks (3 D/F)	8.7791
Error (105 D/F)	5.2211

Mean square required

** (1%)	* (5%)
35.9629	20.5502

the same normal population, and the pooled effect of nutrients becomes a more uniformly weighted effect on the 3 varieties and does not show, as does the analysis of the original data (Table 4), the pooled effect of nutrients to be mostly that within the susceptible variety—that within the resistant varieties being obscured because of the relative magnitude of the differences. Furthermore, the interpretation for interaction is based on a different definition, particularly in relation to varieties. It might now be stated that treatments causing the same proportionate response in different varieties (nutrient × variety interactions) are acting independently of varietal influence.

It should be pointed out, however, that the interpretations of interactions N × P, N × K, and P × K (interactions not involving V) follow rather closely those that would be developed on the nontransformed data, particularly with method D, table 6, with the exception that, in the pooling of variety observations, greater weight is placed on those for the variety showing the least differences on the original scale, and *vice versa.* That is, proportionate treatment within varieties does not alter the relative magnitude of nutrient differences giving rise to nutrient interactions, but it does avoid the contingency of large differences in effects within the susceptible variety, almost completely obscuring the small differences within the resistant variety.

The manner in which this proportionate treatment of the data affects the ultimate result of analysis can be more clearly seen by reference to the graph (Figure 2) of the V × N, the V × P, and the N × P interactions in the original scale and in the scale where the proportionate treatment has been applied. In the analysis of the original data (Table 3) the V × N and the V × P interactions were found significant at odds of 99 to 1, while the N × P interaction failed to attain significance even at odds of 19 to 1. In the analysis of the data following the proportionate treatment (Table 8) the reverse was encountered; here the V × N and V × P interactions failed to attain significance, but the N × P interaction was found significant at odds of 99 to 1. In the graphic representation of these data (Figure 2) it can be seen that where the proportionate treatment was applied the actual wide differences between varieties are eliminated from the statistical interpretation of the treatment and treatment × variety effects. Here V × N and V × P fail to be significant following the proportionate treatment because the treatments cause too nearly the *same proportionate* response within varieties, and N × P interaction is found significant because of the readjustment of treatment differences within varieties. The limited experience with this type of proportionate treatment does not permit a critical evaluation of its usefulness except as a supplementary test. Furthermore, it has been pointed out that P and K effects in this experiment were quite constant and were associated with low specific errors so that partitioning of the error into at least 2 groups, one of which would include all N comparisons, might have been advisable, inasmuch as error variances resulting from other than varietal

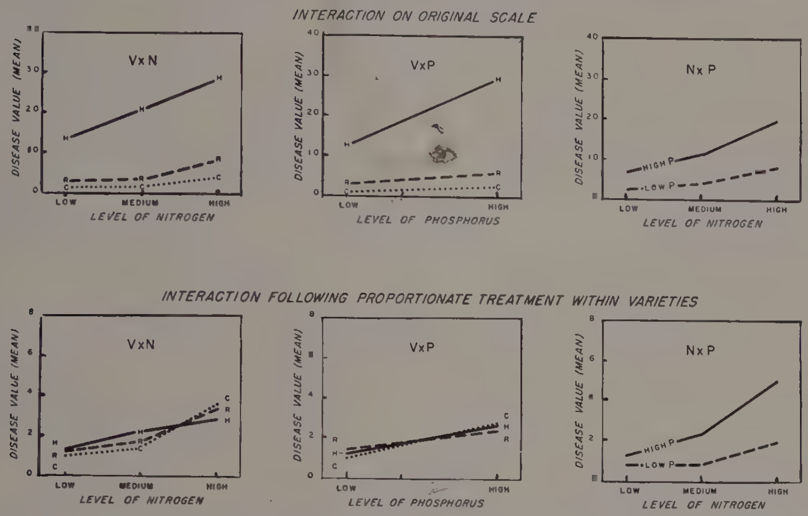

FIG. 2. Graphic representation of the V × N, V × P, and N × P interactions on the original scale and following removal of varietal potentialities for disease resistance by making each observation within a variety a percentage of the total for that variety. Failure of the lines to extend in a parallel direction indicates degree of interaction. Note that V × N and V × P which were highly significant on the original scale have lost significance on the proportionate scale while N × P which was in the doubtful category on the former becomes highly significant on the latter scale.

response have not been equalized. On the other hand, it may be that the chief value of this type of comparison rests in the fact that it enables one to obtain a rough check on the usual algebraic mean values and differences. Thus, an additional analysis of variance may not be warranted.

DISCUSSION AND CONCLUSIONS

It was pointed out in the forepart of this paper that the mean values in table 2 indicated that, at sight, certain main effects could be accepted as significant. Every method of analysis substantiated this conclusion. Consequently, while two of the methods failed to show a highly significant difference between the varieties Cook and Rowden, the main contribution of statistical analysis to this experiment is its aid in studying the interactive effects of the various factors upon wilt development. Since the susceptible variety permits the development of much more intensive disease symptoms than the two more resistant varieties, there is considerable disparity in the magnitude of the errors of observation for different varieties. Hence, variety variances are heterogeneous and may not be validly estimated by the generalized error term. This is also true, but not so pronounced, for nitrogen variances.

Transformations were used to bring about a more normal distribution of the errors of observation, but it was shown (Table 3) that the angular and square-root transformations did not result in homogeneous distributions; whereas the log. transformation not only removed heterogeneity from the data, but also established similarity of proportional response as the criterion of independence. Although there is but little difference in effects attaining significance among any of the 3 transformations employed, the experimenter is handicapped in making a reasonably simple definition for the basis of interactions on the angular and square-root scales. On the other hand, the log. transformation permits an interpretation of interactions on the basis of proportionality of geometric means. Since only one interaction ($V \times P$) attained even the lower level of significance when the data were analyzed subsequent to log. ($\times + 2$) transformation and none on the log. ($\times + 1$), the conclusion to be drawn from this analysis would be that disease development in this experiment was significantly conditioned by all contributing factors, but the interrelationships of the responses to these contributors were, with one possible exception, of reasonably commensurate proportions. Inspection of the means in tables 2 and 7, as well as other methods of analysis, creates doubt as to the validity of these conclusions.

Since the heterogeneity found in these data invalidated the use of the generalized-error term, specific error terms and certain homologous groupings of them were used to test the significance of individual comparisons. This accomplished the essential results for main effects obtained with the most appropriate type of transformation. Attention may be called to the fact that the V_1 comparison (Cook *vs.* Rowden) is not significant when tested by the fully pooled error derived directly from the coded values (Table 4); it reaches the 5 per cent level of significance when tested by its own component of error; and it becomes highly significant when tested by errors based on homologous groups (Table 6) as it also did in the converted data. The writers believe that the indication for high significance for this effect should not be regarded with skepticism.

The interpretation of the interrelationship of the induced variables upon the development of disease, when the individual degrees of freedom are tested by their respective components of error, is to be distinguished from that used for the results based on the logarithmic transformation. Independence in nonconverted data is established by lack of arithmetical disparity in the responses to various factors. In the analysis of the nonconverted data, interactions $V_2 \times N_1$ and $P \times K$ were found to be significant and $V_2 \times P$ highly significant. In other words, the variety Half and Half developed significantly more wilt for given increments in nitrogen and phosphorus supply than did the Cook and Rowden varieties. The decrease in disease associated with increase in K supply was not significantly different in different varieties, but increase in potassium supply does appear to influence the amount of disease developing for a given increment in P supply, ($P \times K$). Lack of significance for all other interactions indicates insufficient difference in disease intensity associated with the various categories of nutrient supply and varietal specificity.

It was pointed out with reference to the logarithmic transformation that the resulting necessity of interpreting the findings in terms of proportionality had a sound biological basis. However, the resulting geometric means are rather awkward to interpret, and some other basis for arriving at proportionality would facilitate examination of mean differences. Data were presented wherein varietal effects were reduced to base levels, and nutritional responses were estimated as a proportion of the total amount of disease observed within a variety. This method of proportional treatment obviated the awkwardness of having to interpret geometrical means that are inherent in the logarithmic transformation. This method also brought out certain relationships within the data that might not have been evident otherwise. This was shown by table 8 and emphasized by figure 2. That is, all varieties showed the same proportionality of disease development as induced by nitrogen and phosphorus nutrition.

Proportionate treatment with varieties as base levels indicated several interactions to be significant that were not found to be so by other methods of analysis, involving, to be sure, different criteria of independence. By way of illustration, $V_1 \times K$ interaction may be considered significant, indicating the proportion of disease development as conditioned by potassium supply differs in Cook and Rowden. That such should be the case is apparent from both tables 2 and 7. The $N \times P$ interaction is found to be highly significant following proportionate treatment within varieties; yet, it is not highly significant on any other scale, although it does reach the 5 per cent level with method D. This means that when varietal specificity is converted to a parity basis, disease development due to nitrogen nutrition is conditioned by phosphorus supply also. One might have surmised such a result from table 2, but the divergence in varietal response so obliterated precision in the estimation of this effect that significance could not be definitely ascertained until varietal propensities were reduced to base levels. One striking characteristic of the results following proportional treatment was the observation of high significance for the $V_1 \times N_1 \times P \times K$ interaction.

This indicates that the different combinations of levels of N, P, and K supply induce a distinctly different proportion of wilt development in the variety Cook as compared with that in Rowden. (This interaction attained low significance when measured by a regrouped error term in straightforward analysis, but little credence was placed upon its validity due to lack of substantiation by parallel methods of testing.) As shown by the significance of interactions $V_1 \times N_1 \times K$ and $V_1 \times N_2 \times K$, the varieties Cook and Rowden differ in the proportionality of their wilt susceptibility as induced by the various combinations of levels of nitrogen and potassium supply. It appears that a distinction in physiological specificity is evident between these 2 semi-resistant varieties. However, it may well be that some of the indications for significance on this basis should be considered as suggestions for future study rather than as established conclusions.

It must be recognized that it is not possible from the foregoing to arrive at any unqualified recommendations as to the method of analysis to use in any given experiment involving pathological symptoms or even to predict that any statistical analysis will greatly improve the suggested interpretation based on inspection of observed results. Most of the discussions in this paper refer to the results from a single experiment, and are not intended as sweeping generalizations. In the present case, analysis was needed primarily to study interactive effects, a contingency that definitely impaired the value of both the angular and square-root transformations. Even though the logarithmic transformation places the relationships on a proportionate basis, the interpretations are complicated by the geometric means involved. Furthermore, the failure of log transformations to indicate significance for certain interactions that other methods of analysis showed to be important and for which, from an inspection of mean values, one might have expected significance, may indicate that the geometric mean inherent in the log scale tends to distort rather than clarify the true relationship of disease development in this experiment. The single degree-of-freedom analysis, using partitioned-error terms, permitted a clear-cut interpretation of the arithmetical differences within the experiment. It seems probable, however, that the interpretation of such differences should be supplemented by a study, or at least an inspection, of differences based on proportionate responses with varieties at base levels.

LITERATURE CITED

1. BARTLETT, M. S. The square root transformation in the analysis of variance. Jour. Roy. Stat. Soc. Suppl. 3(1): 68–78. 1936.
2. BLISS, C. I. The analysis of field experimental data expressed in percentages. Leningrad: Plant Protection Bull. [U.S.S.R.] 12: 67–77. 1937.
3. ————. The transformation of percentages for use in the analysis of variance. Ohio Jour. Sci. 38: 9–12. 1938.
4. BRANDT, A. E. Factorial design. Jour. Amer. Soc. Agron. 29: 658–667. 1937.
5. CLARK, A., and W. H. LEONARD. The analysis of variance with special reference to data expressed as percentages. Jour. Amer. Soc. Agron. 31: 55–66. 1939.
6. COCHRAN, W. G. Some difficulties in the statistical analysis of replicated experiments. Empire Jour. Exp. Agr. 6: 157–175. 1938.
7. FISHER, R. A., and F. YATES. Statistical tables for biological, agricultural, and medical research. Oliver and Boyd (Edinburgh). 1938.
8. SNEDECOR, GEORGE W. Statistical Methods. Collegiate Press, Ames, Iowa. 1937.
9. STEPHENS, W. L. Heterogeneity of a set of variances. Jour. Gen. 33: 398–399. 1936.
10. THARP, W. H. A sand-nutrient infection technique for the study of Fusarium wilt of cotton. Phytopath. 28: 206–209. 1938.
11. WILLIAMS, C. B. The use of logarithms in the interpretation of certain entomological problems. Ann. of Appl. Biol. 24: 404–414. 1937.
12. YATES, F. The analysis of replicated experiments when the field results are incomplete. Emp. Jour. Exp. Agr. 1: 129–142. 1933.

PATHOGENICITY STUDIES WITH ISOLATES OF RHIZOCTONIA SOLANI OBTAINED FROM POTATO AND SUGAR BEET[1]

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INTRODUCTION

In a previous publication,[3] it was reported that isolates of *Rhizoctonia solani* Kühn, obtained from potato, were nonpathogenic to sugar-beet roots. Tests of other isolates of the fungus from potato have been made to determine their pathogenicity to sugar beets, and in certain cases to red garden beets. The isolates of *Rhizoctonia solani* obtained from sugar beets have been tested for pathogenicity to potato. A brief report of some of these studies has been made.[4]

METHODS AND MATERIALS

Unless otherwise noted, the method of inoculation employed with sugar-beet roots was the same as used previously.[5] In the tests with potatoes, the seed pieces were treated for 20 minutes in acid-mercury bichloride prior to planting. In one method, used with potato plants in greenhouse tests, the inoculum consisted of a fungus culture grown on a medium made by autoclaving soaked oats and wheat grains. The inoculum was placed in contact with the underground stem, without wounding, although it is probable that some lateral roots were broken off in removal of soil from around the plants.

A second method was used in certain greenhouse tests and in all field tests with potatoes. For each isolate used, grain inoculum was mixed with steamed soil and allowed to stand about 2 weeks. In the greenhouse, treated seed pieces were placed at the bottom of sterilized 4-in. pots that were subsequently filled with inoculated soil. Attempt was made to maintain a temperature of 16° to 20° C. and to keep the soil moist throughout the experiments.

To inoculate plants in the field tests, small quantities of artificially infested soil were placed in 4-in. paper pots, the treated seed-pieces put in place, then the pots were filled with infested soil. These pots were then buried in the planting furrow in the field. Because of the method used, and from the evidence afforded by check plots, it is thought that infection occurring in the field experiments may, with considerable assurance, be assigned to the isolate incorporated into the soil and not to some organism from the field soil. The potato plants were spaced at 12-in. intervals in rows 28 in. apart.

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[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station, Paper No. 1784 of the Journal Series of the Minnesota Agricultural Experiment Station.

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[3] LeClerg, E. L. Parasitism of *Rhizoctonia solani* on the sugar beet. Jour. Agr. Res. [U.S.] 49: 407–431. 1934.

[4] LeClerg, E. L. Further studies on the parasitism of *Rhizoctonia solani* on sugar beets. Phytopath. 28: 152–153. 1938.

[5] See footnote 3.

The sugar-beet roots used in greenhouse experiments were field-grown roots, stecklings, averaging about ½ lb. in weight. In the field tests, the roots inoculated were about 3 months old and had attained about the same size as the stecklings.

EXPERIMENTAL RESULTS

The potato isolates used were obtained (a) from lesions on underground stems of mature potato plants, the collections being made in August and

TABLE 1.—*Summary of a series of greenhouse tests in which sugar-beet roots were inoculated with Rhizoctonia solani: Isolates from (a) underground stem lesions of potato plants, and (b) sclerotia of potato tubers were compared as to pathogenicity with sugar-beet isolates*

Place from which isolate was obtained	Number of isolates tested	Number of times tested	Total number of sugar-beet roots inoculated	Percentage decay
Control,[a] no organism	–	5	19	0.0
Isolates obtained from potato				
California	3	4	46	0.0
Colorado	4	4	72	0.0
District of Columbia	1	2	13	0.0
Idaho	1	3	20	0.0
Kansas	3	4	44	0.0
Louisiana	1	2	15	0.0
Maine	9	4	145	0.0
Maryland	1	2	11	0.0
Massachusetts	3	4	44	0.0
Michigan	3	5	46	0.0
Minnesota	10	2	150	0.0
Montana	3	5	49	0.0
Nebraska	4	5	70	0.0
New Jersey	1	3	20	0.0
New York	2	3	40	0.0
North Dakota	3	4	55	0.0
Oregon	2	3	31	0.0
Vermont	1	2	31	0.0
Washington	1	3	17	0.0
West Virginia	1	2	11	0.0
Wisconsin	1	2	11	0.0
Wyoming	6	3	77	0.0
Canada	10	4	141	0.0
Denmark	1	3	22	0.0
Germany	3	3	50	0.0
Holland	1	2	13	0.0
Poland	10	3	181	0.0
Isolates obtained from sugar beet				
	8	5	52	54.2

[a] Control plants were treated in same manner as the inoculated plants, except that the grain substratum did not contain any organism.

September in commercial fields, (b) from sclerotia formed on potato tubers, and (c) from badly injured stolons from so-called "blank" hills in commercial fields, isolations being made in April or May. These are referred to as stem-lesion, sclerotial, and stolon-lesion isolates, respectively. In addition to isolations made by the writer, cultures of *Rhizoctonia solani* also have been obtained from a number of plant pathologists in United States, Canada, and Europe.

Inoculation of Sugar-beet and Red Garden-beet Roots

In the previously reported inoculations with sugar-beet roots,[6] 27 isolates of *Rhizoctonia solani* from potato were tested. In the continuation of these tests of pathogenicity, 89 additional isolates from potato were obtained from various points in United States, Canada, and Europe (Table 1). These were used in inoculating a total of 1,425 sugar-beet roots in a series of experiments. Since the isolates from lesions on the underground stems of older potato plants, or from sclerotia on the tubers, in no case caused decay of sugar-beet roots of medium to large size, the results of these tests are summarized in table 1. In each inoculation series, one or more isolates from sugar beet were included, as well as noninoculated plants.

Twenty-three of these isolates from potato, considered representative, together with 6 isolates from sugar beet, were similarly used in inoculations with red garden-beet roots, 5 roots being inoculated with each isolate. The results of these tests are summarized in table 2. The potato isolates were nonpathogenic to red garden beet, whereas the sugar-beet isolates were, under comparable conditions, pathogenic.

TABLE 2.—*Results obtained in greenhouse inoculations of red garden-beet roots with Rhizoctonia solani: Isolates obtained from lesions on underground stems of older plants or from sclerotia on tubers of potato are compared as to pathogenicity with sugar-beet isolates*

Host from which isolates were obtained	Number of isolates tested	Number of plants inoculated	Percentage decay
Potato	23	115	0.0
Sugar beet	6	30	24.7
Control[a]	–	5[a]	0.0

[a] Control plants were treated in same manner as the inoculated plants, except that the grain substratum applied did not contain any organism.

Inoculation of Potatoes with Sugar-beet Isolates

Since none of the isolates of *Rhizoctonia solani* from potato was pathogenic to sugar-beet roots, it was important to test the pathogenicity of sugar-beet isolates to the potato.

A series of preliminary inoculations of potato plants was made using 62 sugar-beet isolates; in these tests, 13 potato isolates were also included for comparison. The underground stems of the potato plants were exposed to the action of the various isolates by the technique previously described. The greenhouse experiment was started November 15, 1934, and was concluded January 8, 1935. A total of 725 potato plants was inoculated with sugar-beet isolates, 73 with potato isolates, and 12 plants were held noninoculated as controls. In recording data, infection caused by an isolate was graded as to severity. The results are summarized in table 3.

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[6] LeClerg, E. L. See footnote 4.

TABLE 3.—*Summary of results obtained with potato in a greenhouse inoculation test using Rhizoctonia solani as represented by 62 sugar-beet isolates and 13 potato isolates: Distribution of the isolates according to severity of infection is indicated*

Host from which isolates were obtained	No. of isolates tested	Distribution of isolates according to degree of infection produced[a]					
		0 to light infection		Moderate infection		Heavy infection	
		No.	Per cent	No.	Per cent	No.	Per cent
Sugar beet	62	10	16.1	15	24.2	37	59.7
Potato	13	9	69.2	4	30.8	0	0.0

[a] Twelve check plants, which were treated in same manner as inoculated plants, except that the grain substratum was used without organism, remained healthy.

From these data it appears that the sugar-beet isolates, as a group, were more virulent to potato than the two types of potato isolates. About 84 per cent of the sugar-beet isolates caused moderate to heavy infection on potatoes; conversely, the potato isolates caused only light to moderate infection. Many of the sugar-beet isolates caused a stem rot that resulted in a toppling over of the affected potato plants.

Another greenhouse inoculation test was made on March 7, 1936, to determine the percentage of emergence of potatoes planted in steamed soil that had been infested with *Rhizoctonia solani* as represented by various isolates. Twelve pots were used for each isolate, and for the control. The cultures used are listed in table 4, and the results of the test summarized. Record was taken on emergence after 1 month. The checks and certain sugar-beet and potato isolates showed perfect stands. It seems safe to attribute the failure of emergence of plants to the attack by the organism used, since sprouts that did not emerge, as sampled, were found on examination to be attacked by *Rhizoctonia.* It is to be noted that several of the sugar-beet isolates were markedly more pathogenic to potato sprouts than the potato isolates used for comparison.

A field experiment was made in the spring of 1935 to test further the pathogenicity of some isolates of the 2 groups of *Rhizoctonia solani* on potatoes, the effects on emergence and height of plants being particularly noted. The results of this experiment, involving 30 sugar-beet isolates and 8 potato isolates, are summarzied in table 5 and illustrated in figure 1. Isolate SB–118 killed all sprouts before emergence; 13 other sugar-beet isolates killed from 4 to 9 of the 12 plants exposed; 4 sugar-beet isolates apparently did not affect emergence, but one of them, SB–84, caused definite stunt-

TABLE 4.—*Effects of sugar-beet isolates and of potato isolates of Rhizoctonia solani on emergence of potato sprouts: Greenhouse experiment started March 7, 1936, in which disinfected tubers were planted in infected soil (twelve 6-inch pots used for each isolate and for the control)*

Host from which isolate was obtained	Place	Designation of isolate	Emergence of plants	
			Number	Per cent
Sugar beet	Michigan	SB–39	12	100.0
do.	do.	SB–42	12	100.0
do.	Manly, Iowa	SB–53	11	91.7
do.	Judson, Minnesota	SB–59	11	91.7
do.	do.	SB–65	11	91.7
do.	Ohio	SB–43	10	83.3
do.	Manly, Iowa	SB–54	9	75.0
do.	Ledyard, Iowa	SB–60	9	75.0
do.	Smith's Mill, Minnesota	SB–69	9	75.0
do.	Elysian, "	SB–74	8	66.7
do.	Glencoe, "	SB–125	6	50.0
do.	Chaska, "	SB–13	5	41.7
do.	East Grand Forks, "	SB–50	4	33.3
Potato	Baarn, Holland	P–100	12	100.0
do.	Baton Rouge, Louisiana	P–116	12	100.0
do.	Dilworth, Minnesota	P–20	11	91.7
do.	Quebec, Canada	P–85	10	83.3
do.	Chwaliszewo, Poland	P–105	10	83.3
Control	....	....	12	100.0

ing of the potato plants; and 4 isolates caused a loss of 1 or 2 plants out of 12. In sharp contrast to this is the record for the potato isolates, which, out of the 8 isolates used in comparison, caused only 50 per cent loss of stand in the most severe cases. Some of the more virulent of the potato isolates apparently caused reduction in height of plants. The evidence in this experiment is judged, as a whole, as indicating that sugar-beet isolates apparently were more aggressive and did greater damage than the potato isolates.

Another field experiment of the same general type, in which potatoes were inoculated with various sugar-beet isolates, was made in 1936. The early loss of stand in this test was small, but toward the close of the season a marked reduction in stand had occurred in plots exposed to certain sugar-beet isolates. Attempt was made to estimate the degree of severity of infection on those plants remaining. These data are presented in table 6. Certain sugar-beet isolates, such as SB–13, SB–43, SB–59, SB–69, SB–101, SB–103, SB–113, and SB–118, markedly reduced stand, and the infection in the plants at harvest was judged as heavy or very heavy. In so far as these isolates were used in previous tests, the record is fairly consistent. Other isolates, such as SB–20, SB–45, SB–49, SB–54, SB–56, SB–77, SB–84, and SB–121, showed light infection of the plants still standing, but some of these, such as SB–20 and SB–45, had severely reduced the stand. Isolates SB–50, SB–68, SB–78, SB–99, SB–111, and SB–128 were classified as medium in severity of infection, but one of these, SB–50, had a consistant record of severe injury to stand in 3 tests. As is to be expected, moderate to very heavy infection of the underground stems generally is to be associated with marked stand reductions. Conversely, those isolates that were not so destructive to stand generally caused less injury to the underground stems.

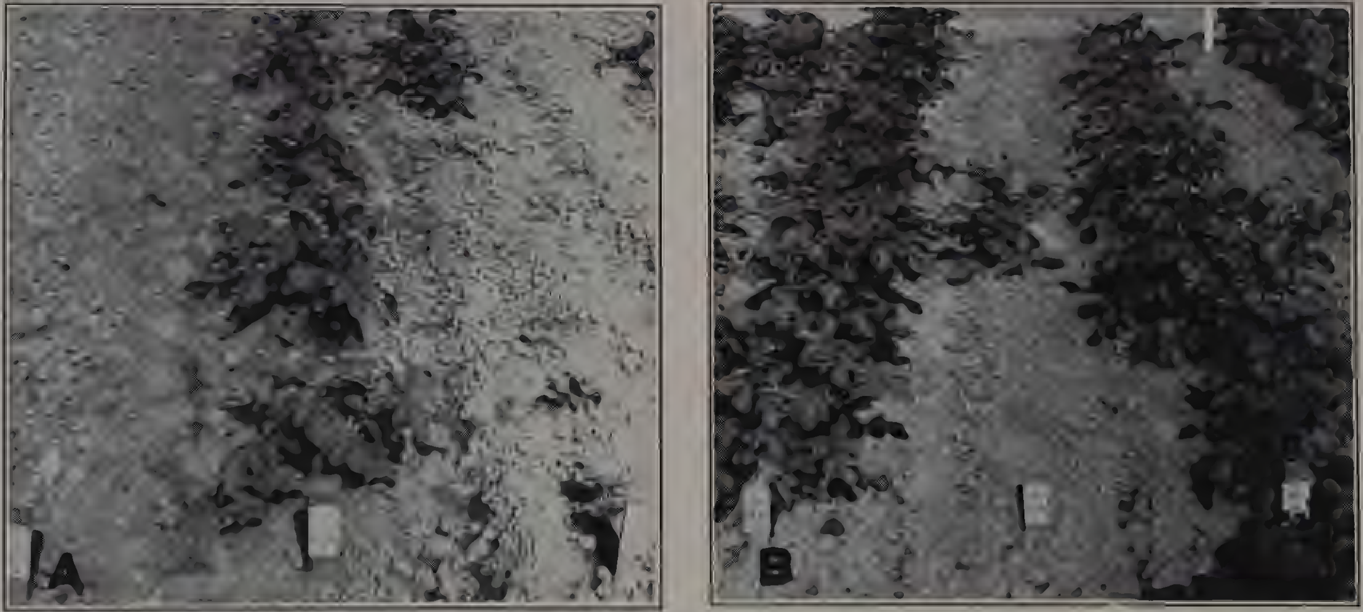

FIG. 1. Comparative virulence of potato and sugar-beet isolates of *Rhizoctonia solani* on potatoes, in the field at St. Paul, Minnesota, in 1938. A, left to right, SB-16, P-18 and SB-78; B, left to right, SB-42, SB-54, and control.

TABLE 5.—*Results of field inoculations of potatoes with sugar-beet isolates and with potato isolates of Rhizoctonia solani: St. Paul, Minnesota in 1935 (12 plants used with each isolate and for the control)*

Isolate[a]	Source	Emergence of plants		Average height of plants in 1935
		Number	Per cent	
				Inches
SB–42	Michigan	12	100.0	18.4
SB–65	Judson, Minnesota	12	100.0	15.8
SB–20	Mankato, “	12	100.0	14.0
SB–84	Carver, “	12	100.0	7.6
SB–52	Manly, Iowa	11	91.7	16.7
SB–64	Judson, Minnesota	11	91.7	13.6
SB–28	Mankato, “	11	91.7	13.4
SB–45	California	11	91.7	12.4
SB–79	Mudbaden, Minnesota	10	83.3	12.4
SB–77	Carver, “	9	75.0	14.1
SB–70	Madison Lake, “	9	75.0	13.2
SB–68	Crookston, “	9	75.0	12.4
SB–125	Glencoe, “	8	66.7	12.2
SB–62	Ledyard, Iowa	7	58.3	14.6
SB–74	Elysian, Minnesota	6	50.0	11.5
SB–39	Michigan	6	50.0	10.3
SB–53	Manly, Iowa	5	41.7	14.0
SB–59	Judson, Minnesota	5	41.7	13.0
SB–78	Shakopee, “	5	41.7	10.4
SB–33	Oslo, “	5	41.7	10.0
SB–50	East Grand Forks, Minn.	4	33.3	10.0
SB–13	Chaska, Minnesota	4	33.3	7.0
SB–43	Ohio	2	16.7	12.0
SB–55	Manly, Iowa	2	16.7	11.5
SB–23	Mankato, Minnesota	1	8.3	15.0
SB–54	Manly, Iowa	1	8.3	15.0
SB–69	Smith's Mill, Minnesota	1	8.3	12.4
SB–60	Ledyard, Iowa	1	8.3	10.0
SB–16	Oslo, Minnesota	1	8.3	3.0
SB–118	Mason City, Iowa	0	0.0	0.0
P–100	Baarn, Holland	12	100.0	16.7
P–85	Quebec, Canada	11	91.7	13.4
P–105	Chwaliszewo, Poland	10	83.3	15.7
P–20	Dilworth, Minnesota	10	83.3	12.0
P–116	Baton Rouge, Louisiana	7	58.3	6.6
P–18	Moorhead, Minnesota	6	50.0	13.2
P–7	Grand Forks, North Dakota	6	50.0	10.8
P–27	Laramie, Wyoming	6	50.0	8.2
Control	....	12	100.0	14.2

[a] SB = sugar-beet isolate; P = potato isolate.

Pathogenicity of Isolates from Infected Potato Stolons to Sugar-beet Roots

The findings of the earlier report and the additional tests here reported showed that none of the isolates of *Rhizoctonia solani* obtained from stem lesions on older plants or from sclerotia on the tubers caused rotting of sugar-beet roots, but, on the other hand, some of the isolates obtained from rotted sugar-beet roots were extremely virulent to potatoes. Fields in many districts are used for both sugar beets and potatoes; hence, in a wide range of isolations from Rhizoctonia-affected potato plants, isolates pathogenic to sugar beets should be encountered.

TABLE 6.—*Results obtained in a field inoculation experiment with potatoes, using sugar-beet isolates of Rhizoctonia solani: 1936 test, St. Paul, Minnesota*

Isolate	Source	Stand percentage on		Degree of infection[a]
		July 7	Sept. 28	
SB–20	Mankato, Minnesota	100	54	L
SB–43	Ohio	100	50	H
SB–45	California	100	62	L
SB–51	Kanawha, Iowa	100	83	VL
SB–59	Judson, Minnesota	100	50	H
SB–68	Crookston, Minnesota	100	96	M
SB–103	Chaska, "	100	58	H
SB–118	Mason City, Iowa	100	29	VH
SB–121	Jordan, Minnesota	100	88	L
SB–49	Michigan	96	96	L
SB–56	Eagle Lake, Minnesota	96	79	L
SB–64	Judson, Minnesota	96	83	M
SB–77	Carver, "	96	88	L
SB–84	Chaska, "	96	79	L
SB–99	Judson, "	96	33	M
SB–111	Ventura, Iowa	96	67	M
SB–54	Manly, Iowa	92	92	L
SB–78	Shakopee, Minnesota	92	79	M
SB–101	Judson, "	92	58	H
SB–69	Smith's Mill, "	88	42	H
SB–113	Garner, Iowa	88	29	VH
SB–128	Ohio	88	71	M
SB–50	East Grand Forks, Minn.	83	33	M
SB–13	Chaska, Minnesota	75	12	H
Control	....	100	100	None

[a] Based on plants remaining alive in the plots: VL = very light; L = light; M = moderate; H = heavy; VH = very heavy.

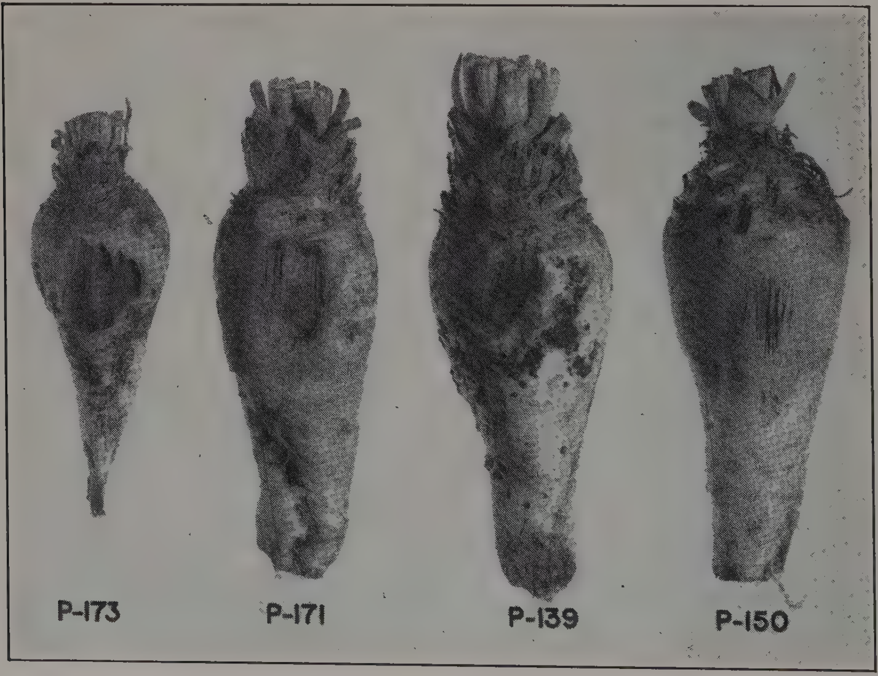

FIG. 2. Type of injury produced on sugar-beet roots by stolon-lesion isolates of *Rhizoctonia solani.* Note the injury on the first three roots caused by P-173, P-170, and P-139 and the absence of injury on the fourth root inoculated with P-150. Each root was wounded with a scalpel before inoculation.

Since the isolates, so far tested, had been limited to lesions on the underground stems of older plants or sclerotia on the tubers, it was thought that isolations from potato stolons infected in the spring might be pathogenic to sugar-beet roots. Accordingly, isolations were made from severely injured potato stolons obtained from ''blank'' hills. Isolates of this type were obtained in the spring of 1935, 1936, 1937, and 1938 in the vicinity of St. Paul, Minnesota; additional isolates were kindly furnished by Dr. L. H. Person, of the Louisiana State University, from collections made near Baton Rouge, Louisiana.

Sugar-beet roots were inoculated in the greenhouse on January 18, 1936, with some of these isolates. The results of the test are given in table 7. Of the 25 stolon-lesion isolates used, 3 caused appreciable decay of the roots, 9 caused slight decay, and 13 were nonpathogenic. Isolates P–162, P–169, and P–151 were the most virulent. Five of the isolates that did not cause typical rot caused a scabbing on the sugar-beet roots at the point of inoculation. A definite depression was usually produced, but the pathogen did not progress beyond this area (Fig. 2).

TABLE 7.—*Results of inoculating sugar-beet roots with Rhizoctonia solani isolates obtained from stolon infections of potato: Greenhouse test started January 18, 1936. (For each isolate, 8 medium-size sugar-beet roots were used)*

Isolate	Source	Average decay	Remarks
	Isolations from potato stolons		
		Per cent	
P–162	New Roads, Louisiana	45.6	....
P–169	do.	41.4	....
P–151	Houma, Louisiana	31.4	....
P–136	Fort Snelling, Minnesota	Slight	2 roots slightly decayed
P–138	Osseo, Minnesota	do.	....
P–139	do.	do.	2 roots slightly decayed
P–148	Houma, Louisiana	do.	....
P–149	do.	do.	....
P–166	New Roads, Louisiana	do.	....
P–171	Brooks Stataion, ''	do.	2 roots slightly decayed
P–174	Baton Rouge, ''	do.	....
P–176	New Roads, ''	do.	....
P–140	Minneapolis, Minnesota	None	Slight scabbing
P–141	Osseo, ''	do.	do.
P–144	do.	do.	....
P–145	do.	do.	Roots scabbed
P–146	Minneapolis, Minnesota	do.	Slight scabbing
P–150	Houma, Louisiana	do.	1 root slightly scabbed
P–152	Baton Rouge, Louisiana	do.	....
P–154	do.	do.	....
P–156	do.	do.	....
P–158	do.	do.	....
P–165	New Roads, Louisiana	do.	....
P–167	do.	do.	....
P–173	Baton Rouge, Louisiana	do.	Roots badly scabbed
	Isolation from sugar beet		
SB–69	Smith's Mill, Minnesota	33.8	....
Control (8 roots)	....	None	....

All of the stolon-lesion isolates tested in the greenhouse in 1936 and several additional ones were used in more extensive field experiments with sugar beets in 1936, 1937, and 1938. In these tests, 97 stolon-lesion isolates were used to inoculate a total of 4,036 sugar-beet roots.

The results of these inoculations are given in table 8. It is significant to note that 6.2 per cent of these isolates caused severe or very severe decay of older sugar-beet roots and 19.6 per cent caused light to moderate decay, 39.2 per cent caused only a small amount of decay, and 35 per cent were nonpathogenic. Thus, about 25 per cent of the stolon-lesion isolates were definitely pathogenic to sugar beets, 11 per cent being moderate to very severe in attack; a sharp contrast to the previous experience of nonpathogenicity of potato isolates.

TABLE 8.—*Classification of 97 potato-stolon-lesion isolates of Rhizoctonia solani on basis of pathogenicity to sugar-beet roots. Field inoculation tests conducted in 1936, 1937, and 1938 at St. Paul, Minnesota*

Grade of infection[a]	Number of roots inoculated	Distribution of isolates on basis of decay produced	
		Number	Per cent
None	918	34	35.0
Very light, tr–5	1,902	38	39.2
Moderate, 6–50	954	19	19.6
Severe, 51–70	171	4	4.1
Very severe, 71–100	91	2	2.1
Total	4,036	97	100.0

[a] For each isolate, a field record was made of invasion of the organism into each root inoculated. From the average of these readings, the infection grade was determined.

RHIZOCTONIA ROOT ROT AS INFLUENCED BY POTATO AND SUGAR-BEET CROPPING

Since some potato-stolon-lesion isolates of *Rhizoctonia solani* were found to be pathogenic to older sugar-beet roots, it was thought advisable to determine the influence of potatoes in cropping systems to Rhizoctonia root rot of sugar beets. Accordingly, field surveys were made in Colorado, Wyoming, and Nebraska in 1936, 1937, and 1938 in areas where potatoes usually precede sugar beets in the rotation. Prevalence of root rot in sugar-beet fields was ascertained by counting the number of affected plants in representative areas of each field. At least 500 plants, and in many cases 600 to 800, were counted.

From table 9, it appears that in each of the 3 years Rhizoctonia root rot tended definitely to be less prevalent in the fields in which the sugar-beet crop followed potatoes rather than sugar beets. Of the total of 50 sugar-beet fields examined in which potatoes were the preceding crop, 82 per cent of the fields show an incidence of root rot less than 5 per cent, with 60 per cent of the fields having only a trace or no root rot. On the other hand, in the 3 years, 28 fields were found in which sugar beets followed sugar beets, and here almost the reverse condition exists in that only 39 per cent of the

TABLE 9.—*Incidence of Rhizoctonia root rot in sugar-beet fields in Colorado, Wyoming, and Nebraska as associated with the previous crop grown,* (a) *potatoes, or* (b) *sugar beets. Field surveys made in 1936, 1937, and 1938 (field averages were determined from counts of 500 to 800 affected plants, various parts of each field being sampled)*

Crop preceding sugar beets	Number of fields examined	Number of fields with indicated amounts of Rhizoctonia root rot of sugar beets						
		None	Trace	1–5%	6–15%	16–25%	25–50%	Over 50%
1936								
Potatoes	15	4	8	2	0	1	0	0
Sugar beets	4	0	0	0	2	1	0	1
1937								
Potatoes	23	7	10	3	1	1	1	0
Sugar beets	14	1	1	6	4	0	0	2
1938								
Potatoes	12	2	4	1	4	0	1	0
Sugar beets	10	0	3	0	3	1	1	2
3-year total								
Potatoes	50	13	22	6	5	2	2	0
Sugar beets	28	1	4	6	9	2	1	5

fields showed 5 per cent or less root rot, with a definite trend away from absence or trace of root rot. It is recognized that there are limits to the conclusions that may be made on results of such a survey, but the data present such contrasts as to suggest strongly the operation of definite effects associated with the cropping practice.

The extreme virulence to potato of many sugar-beet isolates of *Rhizoctonia solani* in the inoculation experiments previously discussed suggested that Rhizoctonia root rot in the sugar-beet crop might lead to severe injury if potatoes were planted the following season.

During the survey of 1936, just referred to, a potato field near Minatare, Nebraska, was examined on August 28 for Rhizoctonia injury. It was ascertained that sugar beets had been grown for 4 consecutive years in this field previous to 1936 and Rhizoctonia root rot had been very destructive in the sugar-beet crop of 1933 and 1934. This field showed a stand of potatoes of about 80 per cent. In the lower portions of the field, the injury from *Rhizoctonia* was severe. Localization of lesions on the stems near the soil surface was commonly noted and production of aerial tubers accompanied the girdling thus produced. The *Corticium* stage of the fungus was abundantly produced on severely affected plants. Isolations P–209, P–210, P–212, and P–213 were made from 4 potato plants taken from this field. Results of subsequent greenhouse pathogenicity tests of these isolates are reported in table 10. Two of these isolates were strongly virulent, causing, respectively, 83.3 and 100 per cent decay of the roots inoculated, whereas the other two were only weakly virulent. This same field was planted to sugar beets in 1937, and was examined by the writer on August 31. About 25 per cent Rhizoctonia root rot was found in the sugar beets, the disease being more prevalent in the lower end of the field where disease in the potatoes had been more severe in 1936.

TABLE 10.—*Results of inoculation of sugar-beet roots in the greenhouse with potato and sugar-beet isolates of Rhizoctonia solani collected at Minatare, Nebraska, in 1936*

Isolate	Number of roots		Average root decay
	Inoculated	Infected	
			Per cent
From potato:			
P–209	9	9	tr.
P–210	9	9	83.3[a]
P–212	9	9	100.0
P–213	9	9	2.8[b]
From sugar beet:			
SB–148	9	9	100.0
Control	9	0	0.0

[a] Roots varied from 40 per cent to total involvement.

[b] Certain roots showed only a trace of decay, considered as 1 per cent in calculation of the average effects.

Interpretation of the high incidence of disease in the potatoes in 1936 as being in considerable part due to carry-over from the affected sugar beets of the previous seasons seemed plausible,[7] especially as potato isolates pathogenic to sugar-beet roots had been obtained from potato-sprout lesions and since the 1937 record shows high Rhizoctonia root-rot incidence in the sugar-beet crop.

Taking into consideration the generally high aggressiveness of *Rhizoctonia* isolates from sugar beet to potatoes, a crop sequence in which potatoes follow sugar beets might be undesirable. The crop sequence in which sugar beets follow potatoes apparently represents a preferable practice and is commonly used in Colorado, Nebraska, and other States where sugar beets and potatoes are grown in the same rotation.

SUMMARY

In tests with 89 isolates of *Rhizoctonia solani* obtained from lesions on the underground stems of older potato plants or from sclerotia on potato tubers, none was found to be pathogenic to sugar-beet roots. Although these isolates were secured from a very wide geographical range, and the results are in line with earlier observations, it is possible that by further sampling, isolates pathogenic to sugar beet may be found.

This point of view finds substantiation in the results of inoculating potatoes with sugar-beet isolates, which, in general, were more aggressive to

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[7] Further evidence of the injury due to *Rhizoctonia* to potatoes following sugar beets is found in a letter received by the writer from Mr. Marx Koehnke of the Nebraska Certified Potato Growers Association. The following statement is taken from this letter: "A year ago I inspected a field (referring to potatoes) that had previously been planted to beets for twenty consecutive seasons. The effect on the potato plants was extreme. Perhaps 50% of the plants were dead or dying, and an even greater number died later in the season. I have seen an occasional field, where small areas in the field had a high infection, and, in fact, practically 100% of the plants would be killed in a spot 15 to 20 feet in diameter."

potatoes than the potato isolates used for comparison. Furthermore, isolates were obtained from potato stolons affected by *Rhizoctonia solani* which in a relatively high percentage rotted sugar-beet roots. From potato plants, grown in a field which had a previous history of sugar-beet cropping and severe root rot, two isolates were obtained from potato stems which were strongly virulent to sugar-beet roots.

Field surveys of sugar-beet and potato fields made in 3 different seasons showed that sugar beets grown following a potato crop were, in general, relatively free from Rhizoctonia root rot, in strong contrast to the situation when sugar beets followed sugar beets. Observational evidence, relative to deleterious effects when potatoes follow a sugar-beet crop in which root rot was a factor, is presented as bearing on the desirability of a potato-sugar beet sequence rather than the reverse order of cropping.

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BROWN–SPOT NEEDLE DISEASE OF PINES

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A serious needle disease known as brown-spot occurs in the several plantations of longleaf pine, *Pinus palustris* Mill., within the Duke Forest. Those who have studied brown-spot (2, 6, 9, 10, 16, 17, 18) regard it as of primary importance to seedlings and young trees. The evidence in hand in the case of the plantations within the Duke Forest, which is located outside the natural range of longleaf pine, indicates that the transplants first became diseased while still in the nursery.

Our special interest in brown-spot began 4 years ago, when there was initiated a study of the morphology and life cycle of the causal organism, best known to forest pathologists as *Septoria acicola* (Thüm.) Sacc. It early became apparent that the pathogen cannot be regarded as a species of *Septoria,* and, furthermore, that it possesses a perithecial stage whose genetic connection was first suspected in 1926 by Dearness (4). The results of these studies are, therefore, herein assembled, and consideration also is given to their significance as applied to dissemination and control.

SUSPECTS AND RANGE

Evidently, brown-spot is not restricted to longleaf pine, but can also involve a number of other species. According to Hedgcock (6) the disease occurs on *Pinus attenuata* Lemm., *P. caribaea* Morelot, *P. echinata* Mill., *P. glabra* Walt., *P. nigra austriaca* Schneid., *P. palustris* Mill., *P. ponderosa* Laws., *P. rigida* Mill., *P. serotina* Michx., *P. sondereggii* Chapm., *P. taeda* L., and *P. virginiana* Mill. The collections available to him were made in Alabama, Arkansas, Florida, Georgia, Idaho, Kansas, Kentucky, Louisiana,

[1] W. V. Brown prepared some of the sections, Mrs. William Millar, figure 3, and we are very appreciative of these kindnesses.

Mississippi, Missouri, North Carolina, Pennsylvania, Oregon, South Carolina, Texas, and Virginia. All observers appear to agree that brown spot is most severe on longleaf pine, especially on seedlings in nursery rows, that it may even constitute a limiting factor in the natural reproduction of this species, and that in the South it is coextensive in range with that of longleaf pine.

APPEARANCE OF THE DISEASE

It may be indicated that longleaf pine seedlings make very little height growth during the first 3- to 5-year period of their existence, but remain as foresters say, in the "grass stage." While in this stage of development each successive season's growth of needles may be killed and remain attached. The oldest dry needles droop around the stem, so clothing it that the seedlings look like tussocks of dead grass, the central portions of which are surmounted by the erect-standing spotted needles (Fig. 1, B).

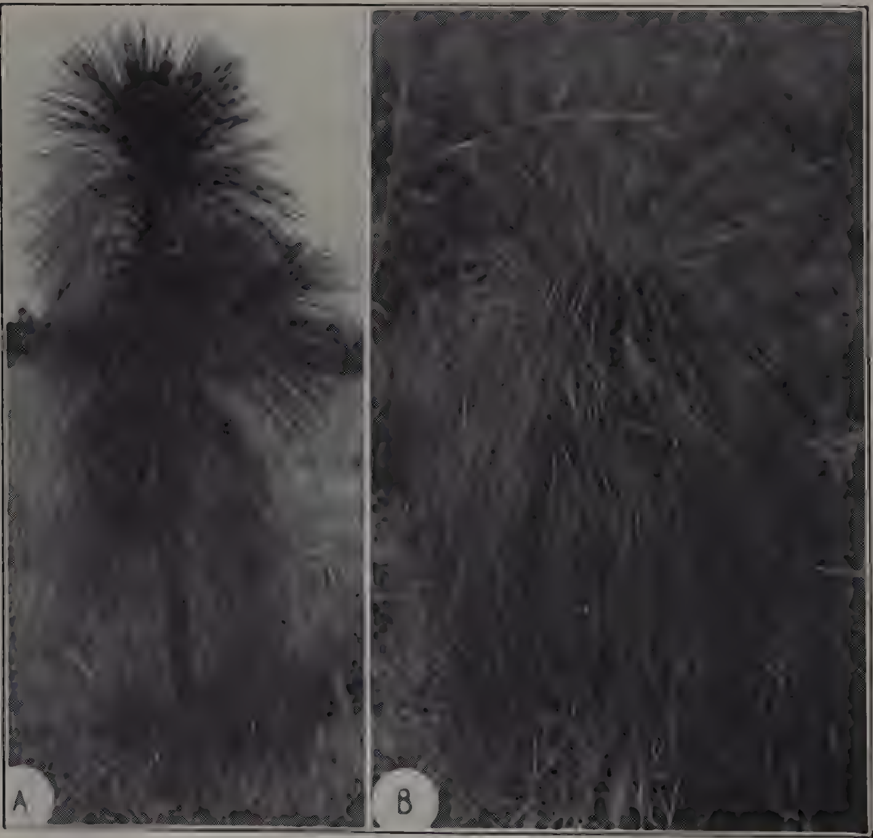


FIG. 1. Comparative appearance of two longleaf pine seedlings of the same age. A. Healthy. B. Stunted by brown spot and still in the "grass stage."

The disease interferes with the growth of the needles, stunting being very marked in case the infection is severe (Fig. 2, A). Ordinarily, the charac-

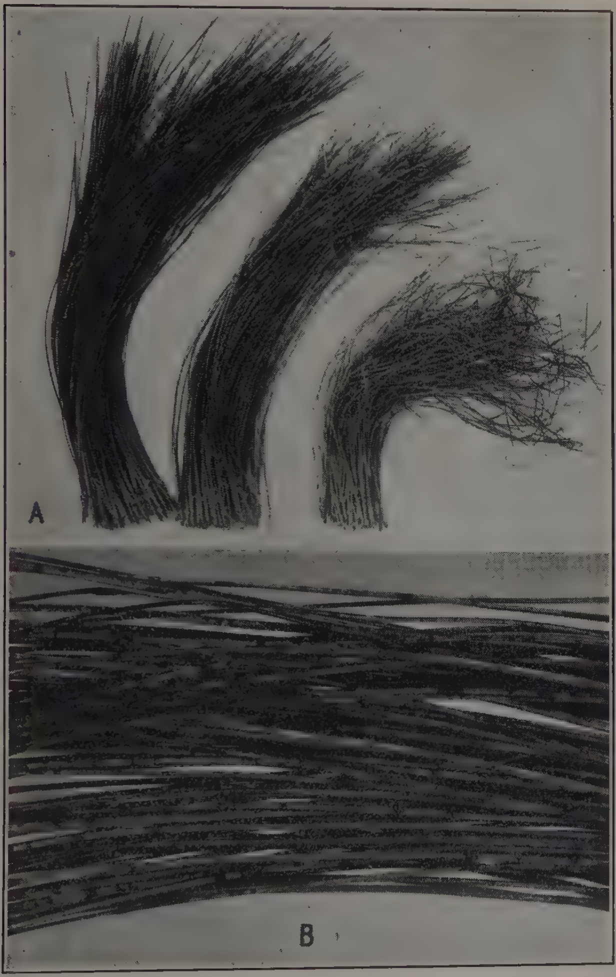


FIG. 2. Groups of longleaf pine needles showing effects of brown-spot. The group at the left in A is composed of healthy needles, those in the middle group exhibit three zones, and those at the right were infected early and are entirely necrotic. Brown-spot lesions on longleaf pine needles, somewhat reduced, in B.

teristic lesions of this brown-spot do not occur throughout the entire length of the needles. Instead, the needles have 3 pronounced zones: (a) the basal part, which is entirely green, above which is (b) the median portion, spotted with scattered lesions that alternate with green tissues (Fig. 2, B); then, above this, zone (c), the dead needle tips. The upper end is usually the first to become diseased because conditions favorable for the spread of inoculum nearly always obtains when growth is renewed in spring or when new needles are developing during summer.

The first evidence of infection is the presence of small, circular spots of yellowish to light grayish-green. Within a few days the tissues nearest the center of these lesions have become brown and necrotic. As the lesions rapidly enlarge they encircle the needle and finally the portion distal to the girdle dies. Within 10 to 14 days after the lesions are first evident, a few dark, elongated pustules, the conidial fruiting bodies, appear. By means of a hand lens it may be noted that they are formed within the leaf tissues and that they emerge by the fissuring of the overlying tissues (Fig. 3, A). By this time the needles may have attained maturity and new infections may have been initiated on that portion of the leaf that is to become the median brown-spotted zone. Evidently, typical lesions cannot be initiated on mature leaf tissues, which may account for the fact that the leaf bases are usually free from brown spots. Once the tissues beyond or between lesions are dead, the mycelium can extend into and ramify throughout them. Needles that are dead throughout most of their length commonly remain attached for a year or more.

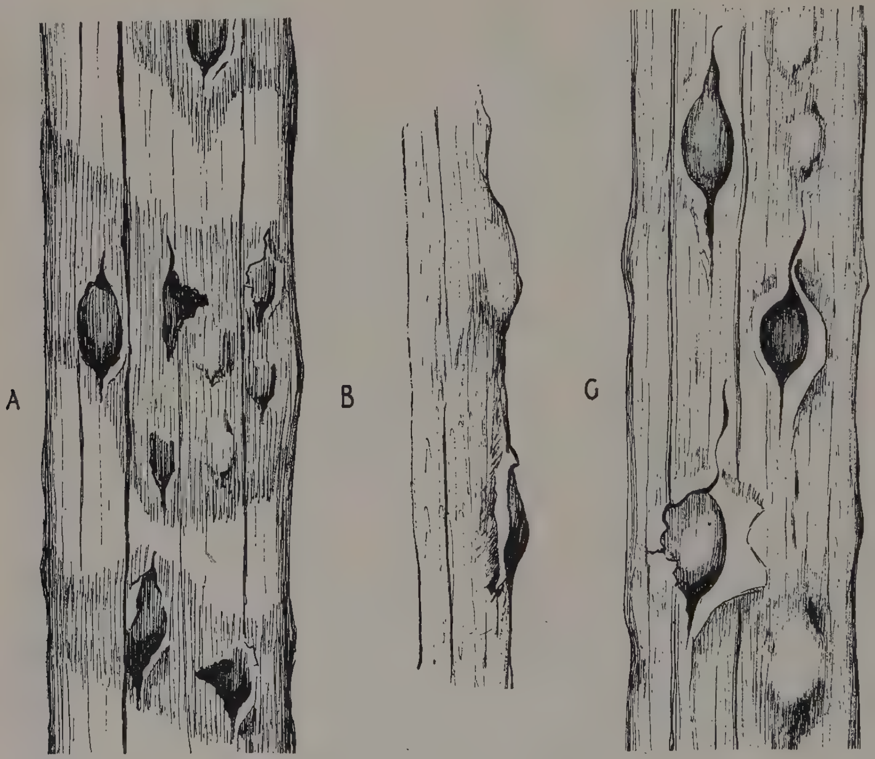


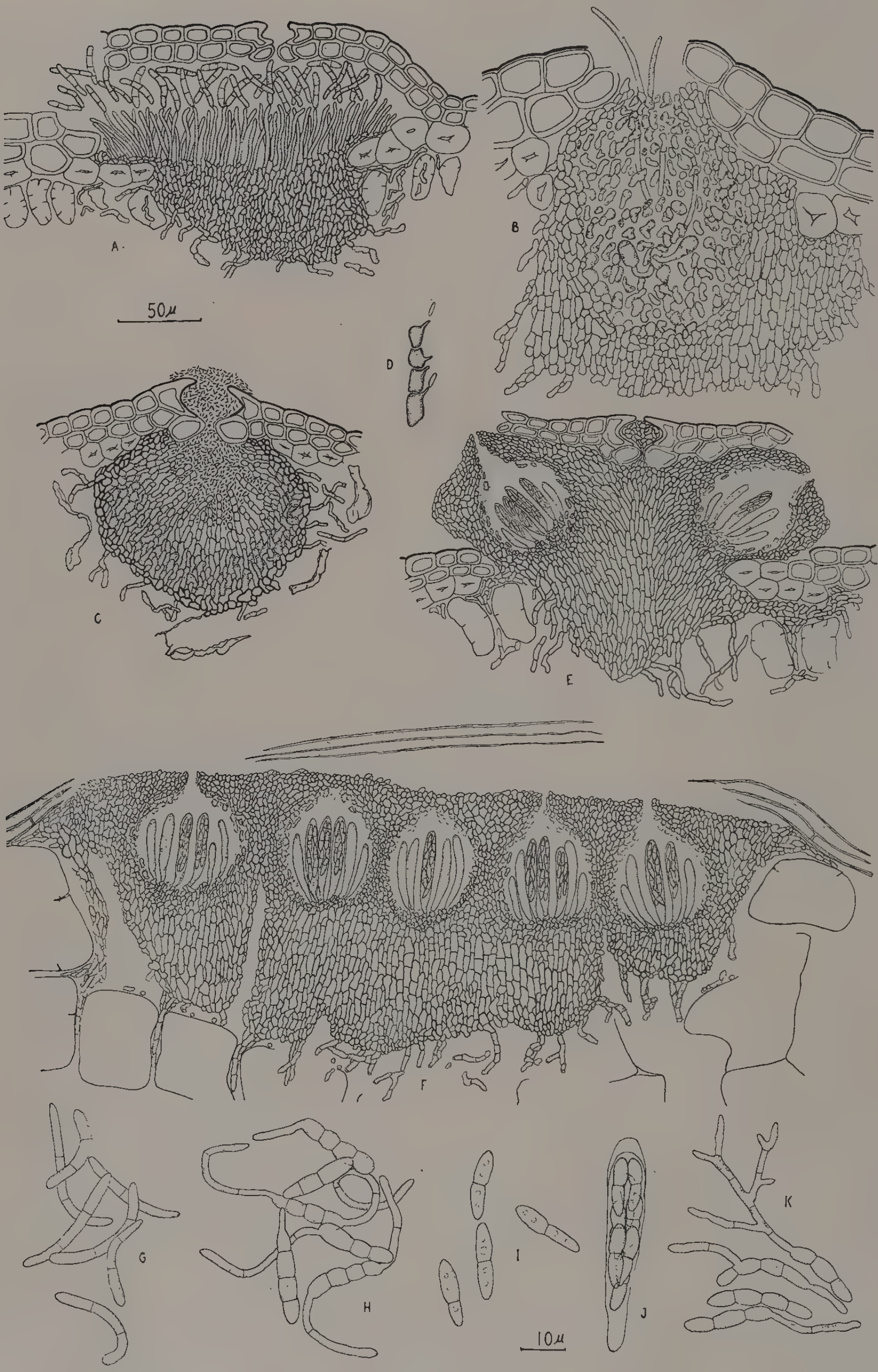
FIG. 3. Sketches of brown-spot lesions as seen with the binocular dissecting microscope. A. Innate-erumpent acervuli in discolored areas with dark conidial heaps. B. Innate-erumpent perithecial stromata as seen projecting above the leaf surface. C. Perithecial stromata as seen from above, either still emersed or having burst the overlying tissues.

If more than one crop of needles is produced during one growing season, each crop may be attacked successively in the same manner. In consequence, growth of the seedlings is retarded and, instead of remaining in the "grass stage" for a few years only before beginning height growth, they may remain in this stage 8 to 10 years, or they may succumb. Quite commonly they are dead by the end of the third season. Chapman (2) observed that in Louisiana 5.3 per cent of longleaf pine seedlings up to 6 inches in height were killed, 34.3 per cent of those 6 to 24 inches tall, and 4.5 per cent of those over 2 feet high. Webster (18) stated that brown-spot is not usually so destructive in Texas, but that, in longleaf pine nurseries, if uncontrolled, it might kill or make unfit for transplanting all of the seedlings in a relatively short time.

DEVELOPMENTAL MORPHOLOGY

To determine the course of development of the pathogen, diseased needles were collected at frequent intervals throughout the year for immediate microscopic examination. Use also was made of series of appropriately fixed, embedded, sectioned, and stained materials. Since the fungus is pigmented throughout, unstained sections were found quite satisfactory for many of the observations. Owing to the fact that longleaf pine needles are heavily cuticularized and possess thick-walled hypodermal fibers, suitable sections were difficult to prepare, longitudinal sections being less difficult, however, than transverse ones.

Although penetration by germ tubes has not been observed, it apparently is accomplished through stomates, since the mycelium is at first localized in the substomatal chambers. In the beginning the mycelium is intercellular involving the mesophyll. After a time, however, the cell walls are penetrated, and become collapsed, their lumina becoming occupied by the hyphae. After the mesophyll intervening between lesions becomes necrotic it may be rather quickly and completely invaded by hyphae. Vascular tissues, however, appear not to be invaded, a fact that may account for portions of the needles, distal to lesions, remaining green for considerable time. Conidial stromata begin to form as soon as the mesophyll tissues become necrotic and the invading mycelium becomes intracellular. They are seated within the mesophyll, generally in the substomatal cavities. These stromata, constituted of rather thick-walled, brown cells, continue to increase in size until they are apparent to the unaided eye as black points entirely submerged within the leaf tissues. At this stage they are button-shape, and on the side toward the exterior of the leaf, which is plane or somewhat discoid, a palisade of unbranched conidiophores 18–25 × 2.5–3 μ is forming (Fig. 4, A). The conidia, which are cylindrical, curved, 1–3-septate (Fig. 4, A andC), and brown, are abstricted seriatim from the apices of these conidiophores. The pressure exerted by the conidia as they accumulate lifts the overlying epidermis and hypodermis and finally ruptures it. Sometimes a lateral cleft (Fig. 4, A) is formed and the overlying tissues remain as a flap partly covering the acervulus. In other cases 2 fissures appear, in which case a strip or flap of host tissue remains above the acervulus. Conidia are extruded from the fissures thus formed. They are held together in a mucoid, water-soluble matrix and, if conditions of moisture are favorable, may


A.
B
50μ
D
C
E
F
G
H
I
J
K
10μ

accumulate in black heaps 500 to 1000 μ in height. Acervuli continue to shed conidia for indefinite periods. Moreover, new acervuli continue to form within necrotic tissues; in fact, viable conidia may be obtained from needles collected at any time throughout the year.

During winter and early spring other dark-color stromata may be found embedded within dead tissues on either or both leaf surfaces (Fig. 3, B and C). These stromata tend to be linear in arrangement and to vary from spherical to elongate-cylindrical. Their dimensions vary from 300–2500 μ in length, 300–500 μ in width and 100–200 μ in thickness. Each stroma contains from a single locule to as many as 10 to 18, arranged in 1, 2, or 3 rows (Fig. 4, E and F).

Eventually, they become erumpent through one or more fissures and protrude above the leaf surface. When such stromata are sectioned they may be noted to contain two types of locules. One type, the spermogonium, is loosely filled with deep-staining cells arranged in columnar manner (Fig. 4, C). These columns extend from the inner periphery of the locules toward the ostiolar orifice. The other type, the carpogonium, is loosely filled with pseudoparenchymatous nurse cells with deep-staining content. Within each carpogonium are one or more archicarps (Fig. 4, B). Each archicarp consists of a basal enlarged portion, the ascogone, above which is a segmented trichogyne that tortuously extends to the exterior.

Apparently, each cell of the columnar chains is spermatiferous. Formation of spermatia first involves those cells nearest the ostiolum. As their content is exhausted their walls disintegrate to the extent of becoming mucoid, and the cells immediately below them are in turn involved. Apparently, a lateral sterigma or papilla is formed from each cell, and the bacilliform spermatia, 2–4 × 1 μ, presumably 4 of which are formed from each cell, are abstricted seriatim from this papilla (Fig. 4, D). Eventually, all the spermatia have been produced and the walls of spermatiferous cells have disintegrated. At this time the locules are filled with spermatia, embedded in a slimy matrix. When moisture is available the spermatia ooze to the surface. Apparently similar structures were first noted by Sydow (12) who states "Wir beobachten auch nicht selten in manchen Gehäusen bald nur ganz vereinzelte bald grossere Mengen von hyalinen, stäbschenförmigen, ca. 2.5–5 × 1 μ grossen Konidien, die den Seitenästen der Träger aufsitzen."

Within 6 to 8 weeks after spermogonia and carpogonia are first found the latter have become transformed into mature perithecia. None of the details

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FIG. 4. Microscopic morphologic details of the brown-spot fungus. A, B, C, E, and F of magnification shown by scale near A. Magnification of D, G to K, that of scale near I. A. Section of acervulus on the conidial stage, *Lecanosticta acicola* (Thüm.) Sacc. on longleaf pine needle. The stroma, conidiophores and conidia, beneath, the ruptured outer layers. B. Section of carpogonial stroma, which is to become transformed into a perithecial locule. C. Spermogonium, with columnar spermatiferous cells, formed in substomatal cavitity. D. Portion of chain of spermatiferous cells, with lateral sterigmata from which spermatia are abstricted. E. Cross section of an elongated perithecial stroma showing in section two perithecial locules. F. Longitudinal section of a mature perithecial stroma showing five perithecial locules. G. Conidia of *Lecanosticta acicola* stage. H. Germinating conidia. I. Ascospores of **Systremma acicola** (Dearn.), comb. nov. J. Mature ascus of *Systremma acicola*. K. Germinating ascospores.

in this process are known but it is highly probable that the spermatia lodge on the trichogynes, effect fertilization, and that the asci arise from the fertilized ascogone. All of the asci in each locule tend to adhere in a fascicle. Not all of them mature simultaneously. Paraphyses are lacking.

The ascus wall consists of 2 membranes. The outer one is thickened apically and is rather inextensible (Fig. 4, K). As a preliminary to ascospore discharge the outer wall ruptures, whereupon the inner extensible membrane elongates in a Jack-in-the-box fashion. In consequence, the ascus tip extends to the outside through the ostiolum, and the complement of ascospores is discharged one at a time in close succession. The empty ascus then is quickly retracted, making room for others in series. The mature ascus measures 35–50 × 7–9 μ. The ascospores are unequally bicellular, the upper cell being the larger, and they are dilutely brown. They measure 15–19 × 3.5–4.5 μ, and each cell commonly contains 2 prominent oil globules (Fig. 4, I).

GROWTH IN CULTURE

This fungus has been grown in pure culture without difficulty, both from conidia and from ascospores. Both the dilution-plate and the streak methods were employed in plantings from suspensions of conidia in water. The conidia germinate readily in water or on nutrient agar, potato agar, or malt agar. As an initial step in germination the cells become swollen and somewhat rounded (Fig. 4, H). Within 24 hours germ tubes have formed from one or more of the cells. Three or 4 days are required before mycelial growth is first evident to the unaided eye, and then only if the Petri dish cultures are viewed in front of a source of light. Gradually, a loose fuscous mycelium becomes apparent and, after 3 or 4 weeks, the colonies have become compact and somewhat stromatoid, are hemispherical, smoky colored to coal-black, and have attained a diameter of 0.3 cm. or less. Within this period, if the cultures are maintained at room temperature, conidia identical with those produced in nature on pine needles will have been formed. Paraffine sections of such colonies show that the acervuli are disc-shaped, some rather flat, others rather deeply concave.

Isolations from ascospores were made by placing pieces of needles bearing mature perithecia on folded paper towels in the tops of Petri dishes. Agar, previously solidified in the bottoms of the dishes, was inverted over these pieces of needles. Under these conditions the ascospores are forcibly expelled onto the agar. Here they germinated (Fig. 4, K) and colonies of the same appearance as those originating from conidia were produced. Moreover, such colonies produce acervuli and conidia, similar in all respects to those originating from conidia. The complete agreement of cultures isolated from conidia with those isolated from ascospores leaves no doubt as to the genetic connection of these two stages.

TAXONOMY

The brown-spot fungus was first named *Cryptosporium acicolum* by de

Thümen (15) in 1878. His specimens, distributed as no. 1484 in *Mycotheca universalis,* constitute the conidial type. These specimens were collected in 1876, at Aiken, South Carolina, by H. W. Ravenel. The host was thought to be *Pinus variabilis* Lamb., but proved to be *P. caribaca* (6). Cryptosporium has innate, acervular fruiting bodies, bearing hyaline conidia, and is among the Melanconiaceae.

In 1884, Saccardo (8), because of the supposed pycnidial nature of the fruiting bodies and septate conidia, transferred it to *Septoria acicola* (Thüm.), among the Phomaceae. Through a typographical error it is included in Martin's (7) list of North American species of Septoria as *Septoria acericola* (Thüm.) Sacc.

In 1918 a collection made at Pike City, Arkansas, was sent to Sydow (12) who, in 1922, being unaware that the pathogen had previously been named, designated it the type of a new genus *Lecanosticta,* among the brown-spore Excipulaceae. He gave it the name *L. pini.* Two years later, after having received additional collections of diseased needles of *Pinus attenuata* and *P. ponderosa* from Idaho and Oregon, and presumably having had access to de Thümen's specimens, he realized that his binomial *L. pini* was untenable. He (13), therefore, changed the name of the brown-spot fungus to *Lecanosticta acicola* (Thüm.), relegating *L. pini* to synonomy.

In 1926, Dearness (4) described *Oligostroma* (*Phyllachora*) *acicola,* among the Phyllachoraceae, noting that it was associated on needles of longleaf pine with *Cryptosporium acicolum* Thüm. Apparently, the type specimens were collected at Silver Springs, Florida, February 27, 1919, by G. G. Hedgcock, his collection number being 32146. Two years later Dearness (5) emended Saccardo's description (8) and pointed out that the conidial fungus on pine needles, while not a typical *Cryptosporium,* fits in this genus better than in Septoria. He also suggested that *Oligostroma acicola* Dearn. may be an ascigerous relative of *Cryptosporium acicolum.*

Hedgcock (6) noted that the fruiting structures of the fungus on pine needles differs from typical species of *Cryptosporium* and of *Septoria;* but, since the fungus resembled species of *Septoria* in artificial culture, he chose to leave it in that genus. This conclusion, as previously stated, met with approval among forest pathologists generally.

Siggers (11), in 1939, having proved by cultural studies the connection of the imperfect and ascigerous stages, transferred this pathogen from *Oligostroma acicola* Dearn. to a closely related genus of the Phyllachoraceae. He denominated it *Scirrhia acicola* (Dearn.), nov. comb.

Most mycologists agree that the Dothideales, as at present constituted, contain two families, the Dothidiaceae and the Phyllachoraceae. These families differ mainly in the ultimate position of the perithecial stromata, those in the former being erumpent at maturity and, in the latter, remaining covered by the host tissues. As has been shown by our studies, the brown-spot fungus produces erumpent, loculate stromata that are at first innate, and, therefore, it must be placed among the Dothidiaceae. In this family, if the generic keys of Theissen and Sydow (14) are followed it fits best in the genus *Systremma* because of the possession of phaeodidymous ascospores and the lack of paraphyses. Since the essential features in the cycle of development of this organism are described for the first time in this report and it appears to belong in the genus *Systremma,* as it is now delimited, the fungus is given the new name **Systremma acicola** (Dearn.), comb. nov. A brief Latin diagnosis, as follows, is therefore given.

Systremma acicola (Dearn.), comb. nov.
Syn. *Crytosporium acicolum* Thüm. Flora 61: 178. 1878.
Septoria acicola (Thüm.) Sacc. Syll. Fung. 3: 507. 1884.
Lecanosticta pini Syd. Ann. Mycol. 20: 211–212. 1922.
Lecanosticta acicola (Thüm.) Syd. Ann. Mycol. 24: 398–400. 1924.
Oligostroma acicola Dearn. Mycol. 18: 251. 1926.
Scirrhia acicola (Dearn.) Siggers. Phytopath. 29: 1076–1077. 1939.

Stromatibus amphigenis, plus minusve lineari-dispositis, sphaericis vel elongatis, innatis, ex mycelio in mesophyllo folii oriundis, late per epidermidem erumpentibus, brunneis vel atrofuscis, 0.3–2.5 × 0.3–0.5 mm. diam.; loculis fertilibus omnino immersis, primum clausis demum ostiolatis, ca. 1–18 in quoque stromate, monostichis v. distichis v. tristichis, ostiolis non-papillulatis, 40–80 μ diam.; asci aparaphysatis, cylindraceo-clavatis, apice obtusis, pariete ad apicem crassiusculis, 35–50 × 7–9 μ, octosporis; sporidiis saepius distichis, inaequaliter 1-septatis, constrictis, loculo supero crassiore, 4-guttulatis, dilute brunneolis, 15–19 × 3.5–4.5 μ.

Spermogoniis et carpogoniis efformantibus, in stromate oriundis, sphaericis, brunneis, 40–80 μ diam.; spermatiis bacillaribus, 3–4 × 1 μ.

Hab. in maculis exardis atque in foliis emortuis Pini spp.

Status conidicus: Statum conidicum *Lecanosticta acicola* (Thüm.) Syd. sistit. Laesionibus fulvellis vel brunneolis; stromatibus acervulorum brunneis v. fuscis, subepidermicis, late erumpentibus et elevatis, punctiformibus vel plus minus lineari-dispositis; sporulis in massulis mucoidis coadunatibus, curvato-lunulatis, cylindraceis utrinque rotundatis, pallide fusco-griseis demum brunneolis, 1–3-septatis, 19–35 × 3.5–4 μ.

Hab. in foliis viridibus et emortuis Pini spp.

For the convenience of mycologists specimens have been deposited in the herbarium of the U. S. Department of Agriculture, the New York Botanical Garden, and the Farlow Herbarium.

GENERAL CONSIDERATIONS

All previous studies of life histories of Ascomycetes possessing a pathogenic conidial stage and a saprogenic ascigerous stage, that have come to our attention, show that the perithecia are initiated in autumn, continue their development throughout the succeeding winter, and mature during spring. It is generally inferred regarding such ascomycetes that low temperature is requisite to perithecial development. The present observations on the brown-spot pathogen indicate that its perithecia may mature at any time within 2 or 3 months after the tissues have become necrotic. Spermogonia and carpogonia, which are the precursors of perithecia, may be first present in March, and, 4 to 6 weeks later, these carpogonia have become transformed to mature perithecia. This difference between *Systremma acicola* and other related Ascomycetes in the season when perithecia are formed may be causally related to two facts, (a) pines are evergreen, several crops of needles being formed within the year, and (b) viable conidia may be present throughout the entire year. These facts suggest that the character of the substrate, *i.e.,* the presence of recently-killed tissues to serve as food, rather than temperature, may constitute the controlling factor in the initiation and development of perithecia of *Systremma acicola.*

Attention may well be called to the fact that the ascospores of *Oligostroma* and *Scirrhia,* to which genera the brown-spot pathogen had previously been assigned, are *hyaline.* If it is recalled that the conidia of the fungus under consideration are brown, that the conidial, spermogonial, carpogonial, and perithecial stromata are composed of cells with dark-brown walls, that the colonies in culture are smoky to black, then one should anticipate brown ascospores. A similar condition, as regards the presence of pigmentation of ascospores exists, as has been shown (19), in the closely related dothidiaceous fungus *Cymadothea trifolii* (Pers.) Wolf, the perithecial stage of *Polythrincium trifolii* Schm. and Kze.

Various mycologists have maintained that the measurements will not reflect the true size of ascospores if desiccated specimens are used. Many Ascomycetes discharge their ascospores immediately after they mature; hence, dried specimens may not contain asci with mature ascospores. Our experience indicates that the size of ascospores can be known only if they are measured immediately after discharge. The measurements of ascospores of the pine brown-spot fungus given by Dearness (4) and by Siggers (11) indicate that they employed immature material.

This organism is admirably adapted to distribution by water. As has previously been mentioned, the conidia are embedded in a mucoid, water-soluble matrix that binds them together in heaps. A suspension of conidia is quickly formed when these heaps are brought into contact with water, and conditions are then ideal for the conidia to be splashed upon near-by objects. Verrall (17), working in Louisiana, pointed out that spattering by rain is primarily responsible for dissemination of the conidia. When he placed slides, smeared with vaseline, sufficiently near diseased pine seedlings to be spattered by rain drops, 100 to 400 conidia per slide were caught. If heavy winds accompanied the rains, as many as 50 conidia per slide were caught 3 feet above the seedlings. Undoubtedly, there is a direct correlation between the height to which conidia are splashed, the relative freedom of seedlings from disease, and the height of seedlings. Verrall (16), on the other hand, has presented evidence that trees more than 5 or 6 feet tall possess a high degree of resistance.

Chapman (2) noted that new infections are almost completely lacking during long dry periods, and that the disease flourishes during wet seasons. According to his interpretation the freedom from infection during dry seasons is attributable to the fact that the fungus "will not survive on foliage at too great distance from the ground where action of wind and sun keep it drier"; but it would seem rather to be the result of lack of production and dissemination of conidia during dry weather.

The ascospores are forcibly expelled in the presence of moisture, as has been stated, and are air-borne. They could be carried by convection currents or by wind to appreciable heights. The likelihood of their lodgment on the foliage and of their being able to initiate infections would be anticipated to decrease rapidly, however, with distance from the ground.

CONTROL

Rather extensive experiments on spraying to control brown-spot in nurseries have been made by Siggers (9). After 2 years' treatment, he found that sprayed seedlings had a diameter 1½ times that of nonsprayed ones in adjacent rows. The fact that conidia may be produced at any season the year around, that they are distributed by rain-splash, that ascospores are formed in dead tissues and are air-borne, and that several crops of new needles may be formed within a year indicate that numerous sprayings would be required to give adequate protection. In view of these things there seems little likelihood that spraying could be done at a cost that would be justifiable and, at the same time, would insure the survival of transplants.

Both Siggers (10) and Chapman (2, 3) have recorded the results of experimentation with fires to control this disease. To the uninitiated this measure might, at first, appear to be sheer folly. It should be borne in mind, however, that longleaf pine possesses great ability to survive fires and that the production of extensive pure stands of longleaf pine were made possible by removal by fires of competing hardwoods. Chapman (3) has pointed out that the natural reproduction of this species is impossible if 3- or 4-years' accumulation of litter lies on the ground. If seed should fall in such litter and should germinate, the seedlings cannot contact mineral soil where conditions of moisture favor their establishment. For this reason he recommends *controlled* burning during the winter prior to seed dispersal. Furthermore, as he states, longleaf pine is intolerant of shade. One or two *controlled* fires, during the period when the seedlings are in the "grass stage" will free them from competition with hardwoods or other vegetation that produces shade. Moreover, the destruction of the brown-spot fungus by these fires has a salutary effect in that it keeps the seedlings sufficiently free from disease so that there is little interference with the initiation of height growth.

After height growth commences, occasional fires are necessary to remove brush and other vegetation that might accumulate to the extent of producing sufficient inflammable material to cause a crown fire that would kill well-established trees, even though 10 or more years old. This hazard is very real in forests from which the ground cover is not removed by occasional *controlled* fires.

In the light of our findings on the life history of the brown-spot pathogen and of its means of dissemination, the successful control of this disease and the regeneration of stands of longleaf pine require, as Chapman (3) advocates, the use of *controlled* fires. Man needs merely to exercise judgment in duplicating the processes that have been in practice for several hundred years, at least long before silviculture and forest pathology acquired the dignity of sciences. If this be done, longleaf-pine forests can be grown and preserved for posterity.

SUMMARY

This study involves the developmental morphology of a fungus causing brown-spot needle disease of pines, especially longleaf pine, *Pinus palustris* Mill. The disease is seriously destructive to seedlings and young trees throughout the entire range of longleaf pine.

Brown necrotic areas form on the needles girdling them. In consequence, the portions distal to the lesions become dry, the needles dwarfed, and the seedlings stunted or killed.

The pathogen possesses a conidial stage, properly identified as *Lecanosticta acicola* (Thüm.) Sacc., and a perithecial stage, *Systremma acicola* (Dearn.), comb. nov. Genetic connection has been established by growth in culture.

Viable conidia may be found at any time throughout the year. The acervuli are innate and become erumpent, the conidia being extruded in a matrix that binds them in heaps. The conidia are adapted to distribution by rain splash to longleaf pines in the "grass stage."

Perithecia are initiated within stromata that bear concurrently both spermogonia and carpogonia. Six to 8 weeks later the perithecia are mature. The ascospores are disseminated by air currents. Presumably, perithecial formation is not controlled by temperature alone.

Since the fungus is dothidiaceous and is pigmented throughout, as established by these studies, it is placed in the phaeodidymous aparaphysate genus *Systremma* and is briefly described.

The brown-spot fungus is indicated from the studies of its life history and dissemination to be best controlled by use of *controlled* fires.

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LITERATURE CITED

1. BOYCE, J. S. Forest Pathology. Pp. 600. 1938. (See p. 98–100).
2. CHAPMAN, H. H. Factors determining natural reproduction of longleaf pine on cut-over lands in La Salle Parish, Louisiana. Yale University School of Forestry Bull. **16**: pp. 44. 1926.
3. ———. The use of fire in regeneration of certain types of forest in India. Jour. Forestry **25**: 92–94. 1927.
4. DEARNESS, JOHN. New and noteworthy fungi. IV. Mycol. **18**: 236–255. 1926. (See p. 251).
5. ———. New and noteworthy fungi. V. Mycol. **20**: 235–246. 1928. (See p. 244–245).
6. HEDGCOCK, G. G. *Septoria acicola* and the brown-spot disease of pine needles. Phytopath. **19**: 993–999. 1929.
7. MARTIN, GEORGE. Enumeration and description of the Septorias of North America. Jour. Mycol. **3**: 37–41. 1887. (See p. 37).
8. SACCARDO, P. A. Sylloge Fungorum. **3**: 507. 1884.
9. SIGGERS, P. V. The brown-spot needle blight of longleaf pine seedlings. Jour.. Forestry **30**: 579–593. 1932.
10. ———. Observations on the influence of fire on the brown-spot needle blight of longleaf pine seedlings. Jour. Forestry **32**: 536–562. 1934.
11. ———. *Scirrhia acicola* (Dearn.) n. comb., the perfect stage of the fungus causing the brown-spot needle blight of pines. Phytopath. **29**: 1076–1077. 1939.

12. SYDOW, H., and F. PETRAK. Ein Beitrag zur Kenntnis der Pilzflora Nordamerikas, insbesondere der nordwestlichen Staaten. Ann. Mycol. 20: 178–218. 1922. (See p. 211–212).
13. ———. Zweiter Beitrag zur Kenntnis der Pilzflora Nordamerikas, insbesondere der norwestlichen Staaten. Ann. Mycol. 22: 387–409. 1924. (See p. 398–400).
14. THEISSEN, F., and H. SYDOW. Die Dothidiales. Ann. Mycol. 13: 149–746. 1915.
15. DE THÜMEN, F. Fungorum americanorum triginta species novae. Flora 61: 177–184. 1878. (See p. 178).
16. VERRALL, A. F. The resistance of saplings and certain seedlings of *Pinus palustris* to *Septoria acicola*. Phytopath. 24: 1262–1264. 1934.
17. ———. The dissemination of *Septoria acicola* and the effect of grass fires on it in pine needles. Phytopath. 26: 1021–1024. 1936.
18. WEBSTER, C. B. Comments on "brown-spot" disease of pine needles in Texas. Jour. Forestry 28: 767–769. 1930.
19. WOLF, FREDERICK A. Morphology of *Polythrincium* causing sooty blotch of clover. Mycol. 27: 58–73. 1935.

PRELIMINARY INVESTIGATIONS ON DWARF BUNT OF WHEAT[1]

C. S. HOLTON

(Accepted for publication June 12, 1940)

INTRODUCTION

The occurrence of dwarf bunt in certain localities in the Pacific Northwest wheat region has stimulated considerable interest and study of this disease in recent years. Although caused by a race of *Tilletia tritici* (Bjerk.) Wint., the dwarf bunt differs markedly from the common bunt caused by other races of *T. tritici* or races of *T. levis* Kühn. It causes excessive dwarfing and tillering of infected plants, has relatively small, hard spore balls (sori), and the chlamydospores of the fungus have unusually prominent reticulations (Fig. 1). The dwarf bunt fungus is further distinguished by the apparent nonviability of the chlamydospores under laboratory conditions, and by the failure to obtain infection by seed inoculation. These distinguishing characteristics of the dwarf bunt were reported by Young[2] in 1935 and Holton and Heald[3] in 1936. Its prevalence in Utah has been reported by Tingey and Woodward.[4] The latter and Holton and Heald[5] also pointed out that this bunt appeared to be more difficult to control than common bunt. Consequently, dwarf bunt presents unique problems both in research and in control. Conditions that favor spore germination, seedling infection, and general development of other known races of *T. tritici* and races of *T. levis* apparently do not favor the dwarf-bunt fungus. These peculiar requirements have necessitated investigations in order to solve these problems. Preliminary results of such investigations,

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station. Published as Scientific Paper No. 453, College of Agriculture and Agricultural Experiment Station, State College of Washington.

[2] Young, P. A. A new variety of *Tilletia tritici* in Montana. (Abstract) Phytopath. 25: 40. 1935.

[3] Holton, C. S., and F. D. Heald. Studies on the control and other aspects of bunt of wheat. Wash. Agr. Exp. Stat. Bull. 339. 1936.

[4] Tingey, D. C., and R. W. Woodward. Relief Wheat. Utah Agr. Exp. Stat. Bull. 264. 1935.

[5] See footnote 3.

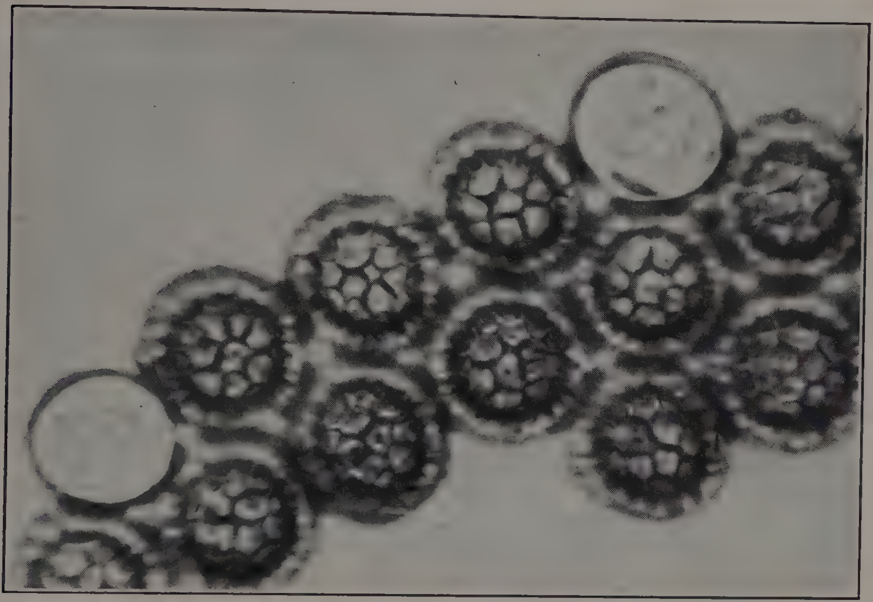

FIG. 1. Photomicrograph showing the prominently reticulate chlamydospores and smooth, hyaline spores of the dwarf bunt.

together with those of studies on the inheritance of certain characteristics of the dwarf bunt fungus, are reported here.

MATERIAL AND METHODS

Chlamydospores of the dwarf bunt used in these studies were obtained from commercial wheat fields in different localities of the Pacific Northwest. Hindi wheat (C.I. 8454),[6] highly susceptible to bunt, was used in the inoculation experiments. The seed was soaked in formaldehyde solution (1–320) for 5 min. and then washed in water to remove the formaldehyde. Inoculations were made with monosporidial lines and combinations of such lines by the method described recently.[7]

The apparent inability of chlamydospores of the dwarf bunt fungus to germinate under laboratory conditions suggested further investigations of this problem. Spores of *Tilletia tritici* and *T. levis* usually have been germinated on 1.5 to 2.0 per cent water agar, and germination usually requires 5 to 10 days, depending upon the physiologic race.

In attempts to germinate dwarf bunt spores the chlamydospores were subjected to various treatments and tested on several kinds of media. Daily observations for germination usually were begun on the fourth day and continued for at least a month. The treatments included alternate freezing and thawing, alternate wetting and drying, incubating at 35° C. for different periods, and treating with Auxilin (Indolebutyric acid). The media on which tests have been made include potato-dextrose agar, water agar, soil-extract solution, soil-extract agar made from raw and baked soil, and juice extract from several varieties of winter wheat. Water agar, in which wheat seedlings were growing and water agar on which chlamydospores of other races of *Tilletia tritici* and *T. levis* were germinating, also was used. Tests also were made by sowing spores on the surface of sterilized and nonsterilized soil kept moist by subirrigation. Tests were made at temperatures ranging from 10° C. to 25° C., and some at 35° C.

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[6] C.I. denotes accession number of the Division of Cereal Crops and Diseases.

[7] Holton, C. S. A simple method of inoculating wheat seedlings with paired monosporidial lines of *Tilletia tritici* and *T. levis*. Phytopath. 28: 518–520. 1938.

EXPERIMENTAL RESULTS

Spore Germination

As yet, no germination of the dark, reticulated, dwarf bunt chlamydospores has been obtained. Despite this failure, sickle-shape secondary sporidia in isolated positions on the agar surface, were rather frequently observed, indicating that germination had occurred, but that the secondary sporidia had been forcibly abjected from the sterigmata of single or fused primary sporidia. However, since germinating chlamydospores could not be detected, it became obvious that the secondary sporidia were arising from some source other than primary sporidia. Numerous smooth-hyaline spores invariably were associated with the regular chlamydospores of the dwarf

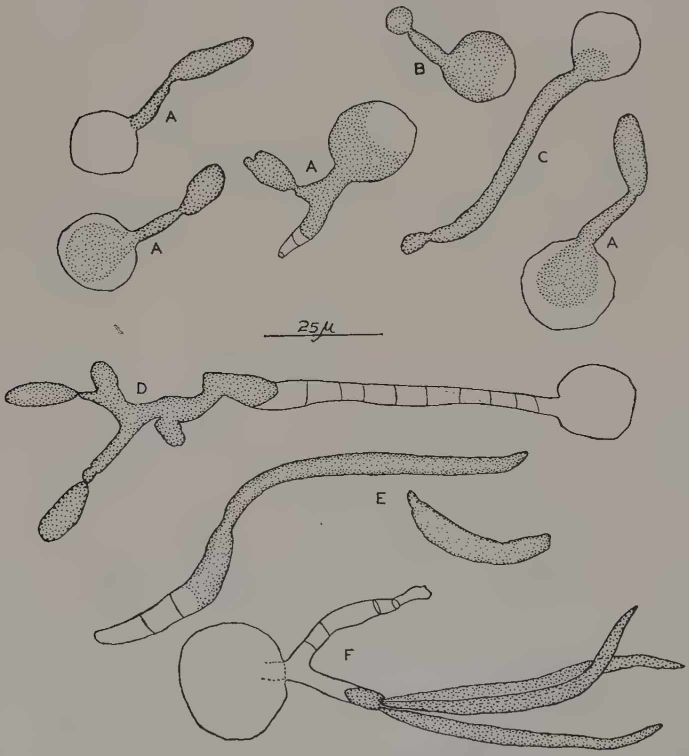

FIG. 2. Camera-lucida drawings of types of germination and the sickle-shape sporidia produced by the hyaline cells associated with the dwarf-bunt chlamydospores.

bunt (Fig. 1). Such spores or cells occur commonly in the sori of *Tilletia* spp., and usually are regarded as sterile or immature chlamydospores. In the germination tests referred to above, however, it was found that some of them had germinated and were producing sickle-shape, secondary sporidia, and also other types of structures. Several types of germination were observed, some of which are shown in figure 2. In some cases, short sterigmata were produced with either sickle-shape or short-cylindric sporidia borne at the tip (Fig. 2, A), the former being forcibly abjected (Fig. 2, E), and the latter remaining attached. In other cases long, promycelium-like structures were formed with a sporidium at the tip (Fig. 2, C). These branched and either developed directly into a mycelium, or produced short sterigmata on which sporidia were borne (Fig. 2, D). The production of 3 typical primary sporidia was observed in one instance (Fig. 2, F).

Probably the most common type of germination observed was the production of a short sterigma-like structure on the tip of which a spherical cell was formed. The stages in the development of the spherical cell from a secondary sporidium were observed and are shown diagrammatically in figure 3. In this case, the hyaline spore produced a sterigma with a sporidium at the tip, which soon began to swell near the center, and finally became

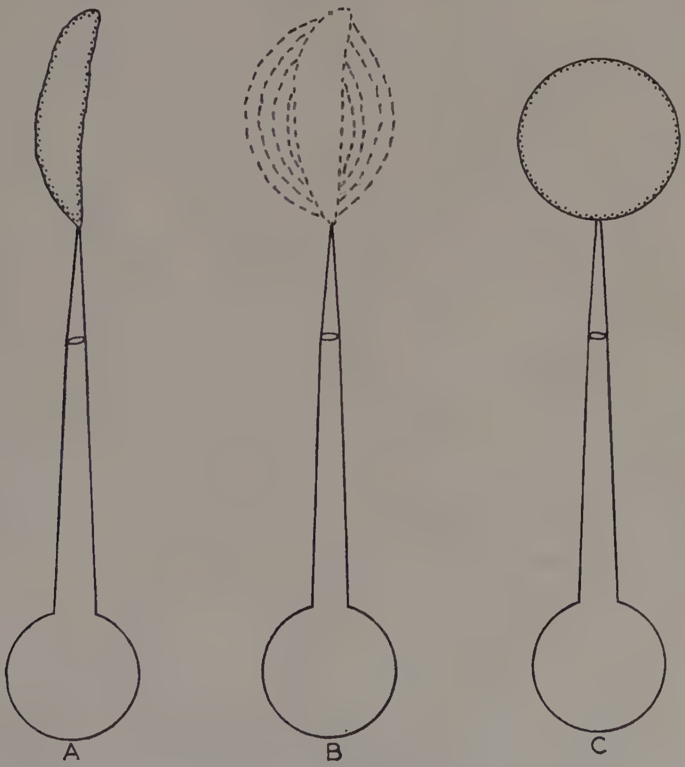

FIG. 3. Diagram showing stages in the development of a spherical cell from a secondary sporidium attached to a sterigma produced by a germinated hyaline spore of the dwarf-bunt fungus.

spherical. These spherical structures normally are aerial and either shrink from desiccation (Fig. 2, B) or explode, spreading cytoplasmic fragments over the agar surface. Invariably, these structures explode if they come into contact with the agar surface, either by being pressed down with a cover slip or by other means. Since the sporidia and sterigmata usually are aerial, the expansion that results in the formation of these spheres apparently is due to absorption of water from the agar through the main spore, or by direct absorption of atmospheric moisture through the wall of the sporidium. Buller and Vanterpool[8] observed a similar type of structure in *Tilletia tritici,* which they believed to be due to a water drop that failed to abject the sporidium and continued to enlarge until the sporidium was enveloped. If the formation of the structures observed and described by the writer was associated with a water drop it seems more likely that the enlargement takes place within the sporidium than that it occurs outside and envelopes the sporidium.

Culture Studies

Cultures of the dwarf-bunt fungus were obtained by inverting a Petri dish of water agar containing germinating spores over a dish containing potato-dextrose agar and allowing it to remain for several hours, during which time the forcibly abjected sporidia dropped to the surface of the nutrient medium below. The sporidia were then located with the aid of the microscope and their positions marked, so that the colonies could be transferred to test tubes when they had developed sufficient size to be visible on the agar surface.

The growth from the sickle-shape sporidia was found to be of 2 distinct types: 1, a haploid type of mycelium which grows rapidly and branches profusely (Fig. 4, A); and 2, a dikaryotic-like hypha, which rapidly increases in length, producing an empty, septate mycelium with only the tip containing cytoplasm (Fig. 4, B). The latter growth continued either to the point of disintegration of the mycelium, or began to branch and produce secondary sporidia and eventually well-developed colonies. Individual branches, however, continue to appear dikaryotic in growth habit. This type of development produces characteristically flat and white colonies. The haploid growth type likewise occasionally produces the flat colony, but in the majority of cases it produces grayish colonies of small diameter and considerable height. In 60 sporidia studied, 47 produced the haploid type and 13 the dikaryotic-like type of growth.

Pathogenicity

No infection was obtained from inoculations with 20 individual lines, indicating that each line was unisexual, notwithstanding the dikaryotic-like type of mycelial development noted above. Infection was obtained, however,

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[8] Buller, A. H. R., and T. C. Vanterpool. The violent discharge of the basidiospores (secondary conidia) of *Tilletia tritici.* Buller, Researches in Fungi, Vol. 5, 416 pp. Longmans, Green and Co. (New York). 1933.

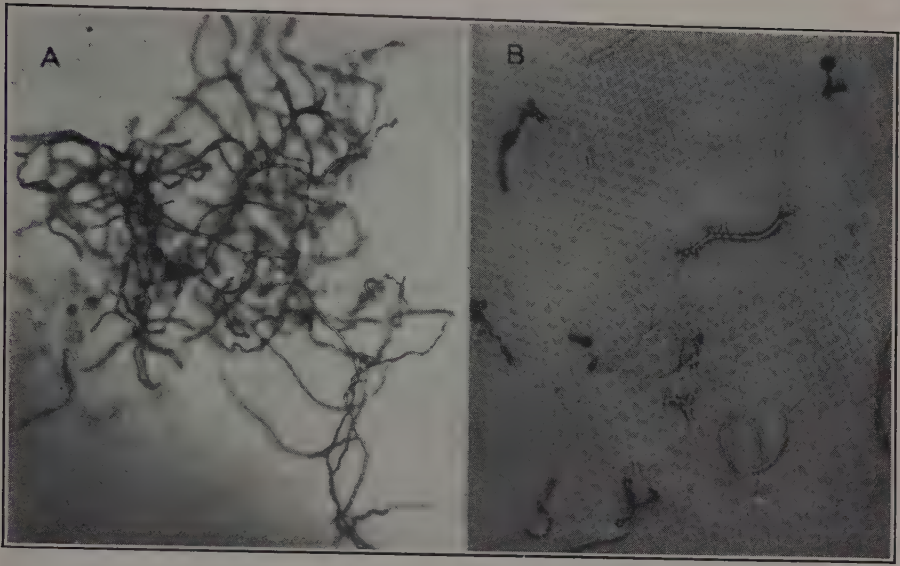

FIG. 4. Two types of mycelium produced by the secondary sickle-shape sporidia of the dwarf-bunt fungus.

from inoculation with 2 combinations of 10 monosporidial lines each, and typical dwarf bunt was produced. The chlamydospores were typical of the dwarf-bunt spores, and only the hyaline cells would germinate.

Repeated attempts to obtain infection by inoculating the seed with chlamydospores and by planting seed in inoculated soil have been unsuccessful, except for occasional heads that probably were the result of infection by germinated hyaline spores. It appeared possible, therefore, that the chlamydospores of the dwarf-bunt race might be nonviable under all conditions and that this disease is being perpetuated by the hyaline spores. For this reason it seemed desirable to determine the percentage of hyaline spores in the total spore population. Counts made at random showed that 8.8 per cent of 1733 spores were of the hyaline type. Their viability has not been determined accurately, but general observations suggest that the percentage of germination is not especially high. Furthermore, inoculation results indicate that the hyaline spores are simply haploid mycelial cells that form a part of the predominantly binucleate (dikaryotic) mycelium and become transformed into resting cells when the diploid chlamydospores are formed. In view of the relatively low proportion of the hyaline spores, the absence of a high percentage of viability, and their apparent unisexual nature, it seems questionable that they are present in sufficient numbers to produce the high percentages of infection occasionally obtained in experiments[9] and frequently observed in commercial wheat fields. On the other hand, if mycelium produced from the hyaline spores is capable of rapid and extensive vegetative development in the soil the above limiting factors would be largely overcome. This possibility is under consideration.

[9] Rodenhiser, H. A., and K. S. Quisenberry. Bunt reaction of some varieties of hard red winter wheat. Jour. Amer. Soc. Agron. 30: 484–492. 1938.

Hybridization

The striking characteristics of dwarf bunt afforded an opportunity for studies of inheritance of ability to produce the characteristic host symptoms, and also of the physiologic and morphologic characters of the dwarf-bunt fungus in crosses between it and other races of *Tilletia tritici* and *T. levis*. Inoculations were made to determine whether the dwarf-bunt race would cross with other races. Infection was obtained with 8 combinations of monosporidial lines of the dwarf-bunt fungus and race 12 of *T. tritici*, and 9 combinations of the dwarf-bunt fungus and race 8 of *T. levis*. In all of the hybrids, the F_1 chlamydospores were reticulate, although the reticulations were less prominent than those of the dwarf-bunt spores. The reticulations on the chlamydospores were more prominent and less variable in size and shape in the hybrids with T12 than in those with L8. In the hybrids studied in the F_2 generation, chlamydospores resembling those of the parents and others with intermediate markings were observed, indicating segrega-

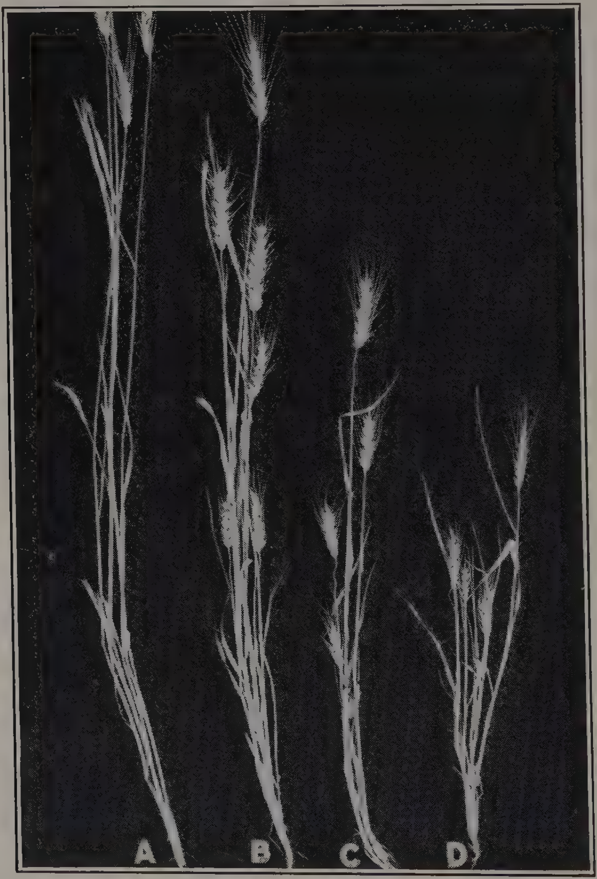

FIG. 5. One healthy and 3 bunted plants of Hindi wheat showing different height classes obtained in plants inoculated with chlamydospores from a hybrid between T12 and the dwarf-bunt fungus: A, Healthy; B, tall; C, intermediate; D, dwarf.

tion and recombination of factors for reticulation and for smoothness of spores.

Germination of the F_1 chlamydospores was obtained in 15 of the 17 hybrids. The viability results were substantiated by those from inoculations with the F_1 chlamydospores. The 15 hybrids with viable chlamydospores produced smut on Hindi wheat, while the 2 hybrids with nonviable spores failed to infect it. It would appear from these results that in hybrids involving the dwarf-bunt race as one parent, viability of the chlamydospores may be dominant in some crosses and recessive in others.

A limited study of the dwarfing capacity of the hybrids showed no definite mode of inheritance for this character. In the F_1 generation, the 8 hybrids with T12 produced 54 smutted plants of which 25 were dwarf and 29 tall. The F_2 spores of these hybrids produced 122 smutted plants of which 31 were dwarf, 45 tall, and 46 intermediate. The F_1 spores of the 9 hybrids with L8 produced 88 infected plants of which 51 were dwarf and 37 tall. In the F_2 generation, these hybrids produced 60 infected plants, of which 10 were dwarf, 41 tall, and 9 intermediate. The behavior in the F_1 generation of both crosses suggests that the cultures were not all homozygous, or that they were not uniform either genetically or in their tendency to produce dwarfness. The classification of smutted plants as dwarf, intermediate, or tall (Fig. 5) is more or less arbitrary, although the plants were separated into the 3 classes rather readily. It would appear from these results that there was segregation of factors for dwarfing. However, it must be recognized that plant height and the expression of dwarfness may vary considerably, depending both upon environmental factors, and upon the uniformity of the bunt cultures. Further studies on segregation for this character are being conducted on winter wheats.

SUMMARY

The reticulate chlamydospores of the dwarf-bunt race of *Tilletia tritici* failed to germinate under all laboratory treatments tried, but the smooth, hyaline, haploid resting spores, occurring with the reticulate chlamydospores, often germinated.

Monosporidial lines of the dwarf-bunt race were obtained by isolating sickle-shape, secondary sporidia produced by the hyaline spores. Some of the single sporidia produced a haploid type of growth, while others produced an apparently dikaryotic type of mycelium, but the former type predominated.

The unisexual nature of the monosporidial lines is indicated by the infection obtained with combinations of monosporidial lines and the lack of it with individual lines. Infection resulted in host symptoms and chlamydospore characteristics typical of dwarf bunt.

Hybrids between the dwarf bunt race and race 12 of *Tilletia tritici* and race 9 of *T. levis* were obtained by inoculating with combinations of mono-

sporidial lines. Evidence was obtained of segregation for chlamydospore morphology and for the dwarfing of the host.

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ASSOCIATION OF STEREUM MURRAYI WITH HEART ROT AND CANKERS OF LIVING HARDWOODS

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INTRODUCTION

In a recent paper, "The Genus *Stereum* in Pennsylvania," Overholts (8) states that 4 species of the genus are known to be important in decay of heartwood of living trees: *Stereum frustulosum* (Pers.) Fr., *S. gausapatum* Fr., *S. sanguinolentum* Alb. and Schw., and *S. subpileatum* B. and C. With the exception of *S. subpileatum,* which was reported by Long (6), in 1915, as causing a heart rot of oaks, the importance of these species as heart-rotting fungi in North America has been demonstrated rather recently by Faull and Mounce (5), Roth and Sleeth (10), and Davidson (3). A fifth species, *S. murrayi* (B. and C.) Burt, has been isolated occasionally by the writers from the decayed tissue of living hard woods. Lorenz and Christensen (7) reported it from the Lake States, and it has also been found in Pennsylvania, New York, and the New England States.

SPECIES ON WHICH STEREUM MURRAYI OCCURRED

Burt (2) states that *Stereum murrayi* fruits "on rotting logs and limbs of frondose species such as *Acer, Betula, Fagus, Quercus* and *Tilia.*" Overholts (8) mentions it as occurring on "dead wood of deciduous trees but especially common on *Betula* and *Ostrya.*" The writers observed it fruiting most frequently on rotting yellow birch (*Betula lutea* Michaux) logs and collected it occasionally on dead trunks or branches of sugar maple (*Acer saccharum* Marshall), apple (*Malus malus* (L.) Britten), and ironwood (*Ostrya virginiana* (Miller) Koch). Sporophores associated with cankers were collected on striped maple (*Acer pennsylvanicum* L.), beech (*Fagus grandifolia* Ehrhart) and ironwood. The fungus was isolated from the margin of a canker on one yellow birch and 15 times from heart rot in merchantable trees of the same species. One isolation was obtained from a canker on sugar maple and one from decay in living red maple (*Acer rubrum* L.). Two isolations were obtained from decayed paper birch (*Betula papyrifera* Marshall). Table 1 gives in detail data concerning the various collections and isolations of *S. murrayi.*

CANKERS ASSOCIATED WITH STEREUM MURRAYI

Stereum murrayi cankers on living trees (Fig. 1, A to C) were generally elongated with depressed centers and swollen margins and were associated

TABLE 1.—*Data on various collections and isolations of Stereum murrayi*

Species	Sporophores on			Isolations from			
	Dead logs or limbs	Cankers on living trees		Cankers without sporophores on living trees		Heartwood of living trees	
	Area	Trees infected	Area	Trees infected	Area	Trees infected	Area
Acer pennsylvanicum		1	White Mts. Nat. Forest, N. H.				
Acer rubrum						1	Peru, Vt.
Acer saccharum	Lake States, Pennsylvania New Hampshire			1[a]	Ottawa Nat. Forest, Mich.		
Betula lutea	Lake States, Pennsylvania New York New England States			1[a]	Nicolet Nat. Forest, Wis.	2	Loretta, Wis.
						3	Jump River, Wis.
						7	Park Falls, Wis.
						1	Rhinelander, Wis.
						2	Ottawa Nat. Forest, Mich.
Betula papyrifera						2	Minnesota
Fagus grandifolia		2	Green Mts. Nat. Forest, Peru, Vt.				
Malus malus	Peru, Vt.						
Ostrya virginiana	North Lawrence, N. Y.	1	Nicolet Nat. Forest, Rhinelander, Wis.				
		1	Tunxis State Forest, Conn.				
		1	North Lawrence, N. Y.				

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[a] Obtained in the course of routine isolations from cankers of various kinds.

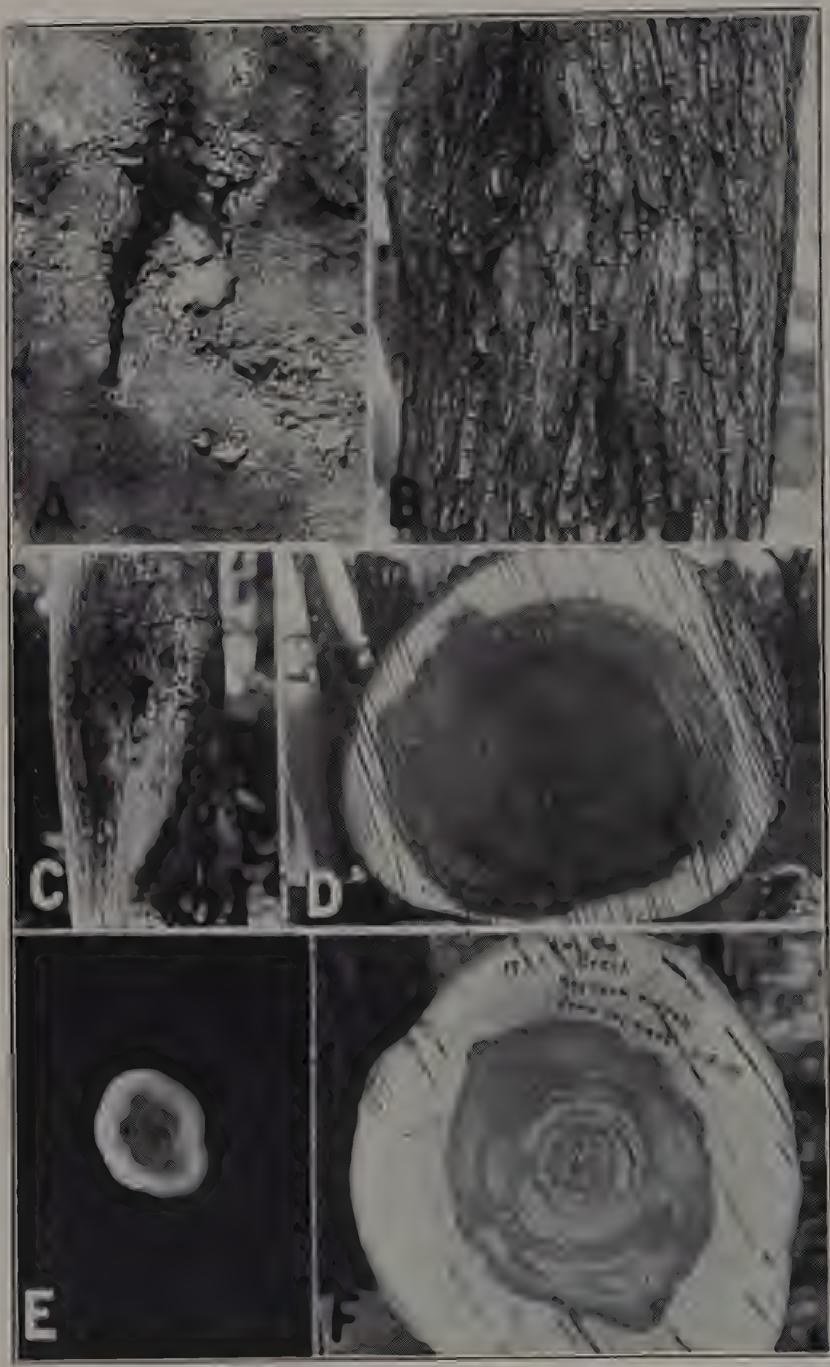

FIG. 1. A to C. Cankers on living trees with which decay and sporophores of *Stereum murrayi* were associated. D and F. Cross sections of decayed trunks. A and F. Beech; B and D. Ironwood; C. Striped maple. E. Fourteen-day-old culture on malt agar at room temperature.

with decay. Such cankers were accompanied by considerable distortion of the trunk, which rendered them conspicuous, especially if several occurred on the same tree. The fungus, in the few cases observed, entered dead branch stubs or injuries and caused cankers by invading the surrounding cambium and sapwood. On most of the infected trees additional cankers had formed at branch stubs or branch traces by the outward growth of the fungus from the decayed heartwood. Sporophores of *Stereum murrayi* developed either on the margin of the older cankers or on the central portions of the relatively young ones. White masses of mycelium were usually present in the inner bark of the young cankers.

EXTENT AND TYPE OF DECAY

In most of the *Stereum murrayi* infected trees the longitudinal extent of the decay was not recorded. A cankered beech, 13 in. d.b.h., was dissected and found to be decayed 4 feet above and 2½ feet below the cankers, which were at heights of 3 and 4 feet from the ground. A cankered striped maple (5 in. d.b.h.) was dissected and found decayed from approximately ground line to a height of 15 feet. There were 5 well-defined cankers at different heights, 2 large ones at 4 feet, and smaller ones at 8, 10, and 12 feet. One ironwood had numerous cankers on the trunk and was decayed from the base to approximately 16 feet. Another ironwood with a single large canker was decayed approximately 3 feet above and 3 feet below the canker. Decay samples from yellow birch, which yielded *S. murrayi,* were obtained from the basal portions in some cases and from the upper trunk region in others.

The decay, which had a distinctive "sweet" odor, was usually confined to a central cylinder of discolored wood (Fig. 1, D and F) and advanced longitudinally much more rapidly in the central part of the discolored area than at the outer margins. Ordinarily, *Stereum murrayi* decay was not associated with trunk swellings except where cankers had formed. Advanced decay was white or light-brown, but the incipient stages commonly merged into the dark-brown of the heartwood or discolored central cylinder. Sometimes restricted areas or narrow channels of advanced decay were visible in areas of otherwise incipient decay but there was no sharp line of demarkation between decayed and sound wood.

STEREUM MURRAYI IN CULTURE

Stereum murrayi is readily obtained in pure culture from both incipient and advanced stages of decay in living trees or rotting logs. In this respect it differs strikingly from a number of other heart-rotting fungi, such as *Fomes connatus* (Weinm.) Gill., which are often inhibited in culture by the ever present non-decaying fungi and bacteria. Occasionally, *Torula ligniperda* (Willk.) Sacc., one of the most prevalent organisms in red heart of birch, was observed growing in the substratum at the edge of the heavy white surface mat of *S. murrayi,* but usually pure cultures were obtained in spite of the slow growth of the fungus.

Detailed Descriptions of Cultures

Petri-dish Cultures.—Growth very slow, forming in 7 days on 2.5 per cent malt agar a chalky white, heavy, compacted, velvety, somewhat appressed mat, 1 cm. or less in diameter; margin white, even.

In 14 days, mat 1.5–2.0 cm. in diameter at room temperature averaging 26° C. (Fig. 1, E), compacted, white to cream color, velvety or short cottony, appressed, heavy, at times slightly zonate and often with pronounced furrows from margin to inoculum; margin very definite, white, even.

Hyphae uniformly 2–4 μ in diameter, with clamps; no secondary spores; swollen or inflated cells, which correspond to the vescicular cells found in the sporophore, are usually present in the cream-color portion about the inoculum.

Strong positive reaction with tannic acid medium (4).

Temperature Relations.—Optimum temperature for growth approximately 25° C. Average diameters of mats in 7 days, in dark, at constant temperatures, as follows: 1.5 cm. at 20° C.; 2.5 cm. at 25° C.; no growth at 30° C. or above.

Test-tube Cultures.—Owing to the extremely slow rate of growth, the mat in 4 weeks is confined to the agar slant and is thick, compacted, white to cream color, often much furrowed and ridged. Agar under mat russet-vinaceous to fawn color (10). Odor prominent, penetrating, described by Badcock (1) as "vanilla."

SUMMARY

Isolations from decay in living yellow and paper birch showed *Stereum murrayi* to be one of the heart-rotting fungi occasionally present. Isolations were obtained from a canker on yellow birch and one from sugar maple. The fungus was isolated also from incipient decay in red maple. Sporophores were found associated with trunk cankers and heart rot of ironwood, beech, and striped maple.

S. murrayi was easily obtained in pure culture from both advanced and incipient decay and could be identified by its characteristic slow growth, dense white mycelium, distinctive odor and the russet vinaceous to fawn color imparted to the agar under the mat.

S. murrayi apparently enters living trees through dead branch stubs, or injuries either near the base or in the upper trunk. Survey of fungi causing decay in northern hardwoods is not sufficiently advanced for a definite statement of the relative importance of *S. murrayi* as compared with other decay species but on some areas in the Lake States where cull studies were initiated it was occasionally isolated from heart rot of merchantable yellow birch.

BUREAU OF PLANT INDUSTRY AND
CIVILIAN CONSERVATION CORPS,
UNITED STATES DEPARTMENT OF AGRICULTURE

LITERATURE CITED

1. BADCOCK, E. C. Preliminary account of the odour of wood-destroying fungi in culture. Trans. British Mycol. Soc. 23: 188–198. July, 1939.
2. BURT, E. A. The Thelephoraceae of North America. XII Stereum. Ann. Missouri Bot. Garden 7: 81–248. 1920.
3. DAVIDSON, R. W. *Stereum gausapatum,* cause of heart rot of oaks. Phytopath. **24**: 831–832. 1934.
4. ———, W. A. CAMPBELL, AND D. J. BLAISDELL. Differentiation of wood-decaying fungi by their reaction on gallic or tannic acid medium. Jour. Agr. Res. [U. S.] 57: 683–695. 1938.
5. FAULL, J. H. AND IRENE MOUNCE. *Stereum sanguinolentum* as the cause of "sapin rouge" or red heart rot of balsam. Phytopath. **14**: 349–350. Abstract, 1924.
6. LONG, W. H. A honeycomb heart-rot of oaks caused by *Stereum subpileatum.* Jour. Agr. Res. [U. S.] 5: 421–429. 1915.

7. LORENZ, R. C. AND C. M. CHRISTENSEN. A survey of forest tree diseases and their relation to stand improvement in the Lake and Central States. U. S. Dept. Agr. Bureau Plant Industry. Mimeograph. 1937.
8. OVERHOLTS, L. O. The genus *Stereum* in Pennsylvania. Bul. Torrey Bot. Club **66**: 515–537. 1939.
9. RIDGWAY, ROBERT. Color standards and color nomenclature. 43 pp., 53 color plates (Washington). 1912.
10. ROTH, E. AND B. SLEETH. Butt rot in unburned sprout oak stands. U. S. Dept. Agr. Tech. Bul. 684. 1939.

THE RHIZOCTONIA FOOT-ROT OF ANNUAL STOCKS (MATTHIOLA INCANA)

A. W. DIMOCK

(Accepted for publication June 20, 1940)

Rhizoctonia foot-rot of annual stocks (*Matthiola incana*) has long been an important source of loss to commercial cut-flower growers in the Eastern States. During the past 2 years the writer either has seen the disease or received reports of its occurrence in the majority of the New York cut-flower establishments in which stocks are grown. Losses may be restricted to a small percentage of the total planting, but it is by no means uncommon to see 50 per cent or more of a crop destroyed. In spite of its seriousness and widespread occurrence, the disease has apparently received little attention from pathologists. Peltier (6) briefly noted the occurrence of Rhizoctonia damping-off of stock seedlings in commercial greenhouses in 1914, while Balfe (4), in 1935, described the disease more fully and offered proof of the pathogenicity of Rhizoctonia (*Corticium vagum*). She discussed only the seedling phases, however, and did not state whether older plants were or were not affected. Other references to the disease include reports of a stem rot of stocks in New Jersey attributed to *Rhizoctonia solani* (1), a root rot of stocks caused by *Corticium vagum* in Mississippi (2), and a *Rhizoctonia* foot and stem rot of stocks in New Jersey and California (3). While these reports apparently refer to the disease on mature plants, they merely record its occurrence and include no descriptive material.

SYMPTOMS OF FOOT ROT

Stocks may be affected with foot rot in all stages of development, though symptom expression varies considerably with the size of plant involved. Small seedlings usually damp off within 2 or 3 days after being set in infested soil. Slightly larger plants, up to 3 or 4 inches tall, may wilt completely, but remain green and erect, even though completely girdled at the soil line. Symptoms on such plants bear a marked similarity to those described for wire stem of cabbage. When older plants become affected, the first symptom is a yellowing of the lower leaves, followed in due course by complete wilting of the foliage (Fig. 1). The small leaves in the unfolding buds of both small and large plants usually take on a bluish hue and remain turgid long after the older foliage has wilted.

Examination of the stems of affected plants reveals the presence of girdling lesions at or just below the soil line (Fig. 2). The affected tissues

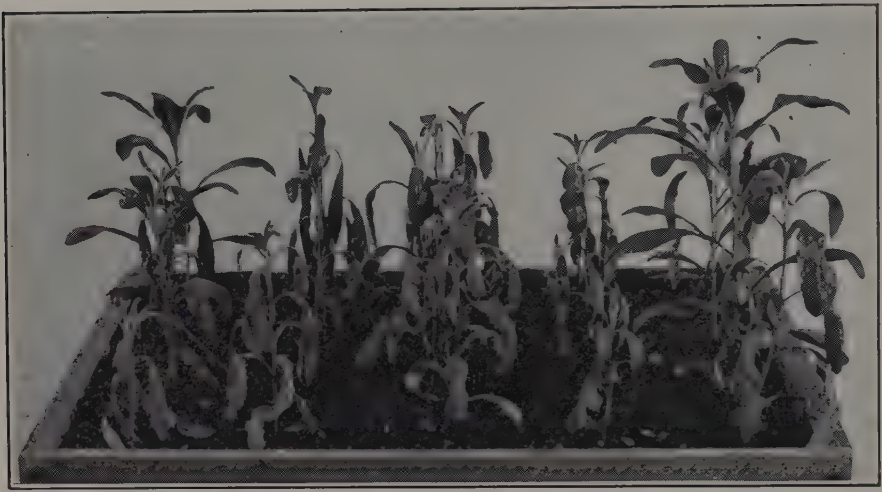

FIG. 1. Rhizoctonia foot-rot symptoms on annual stocks. Seedlings set in sterilized soil November 13; flat inoculated January 15; first symptoms January 22; photographed January 26.

turn black and become markedly constricted. Although infection almost invariably occurs at the soil line, the entire root system may eventually be involved. Quite often lesions are at first restricted to one side of the stem, which leads to one-sided development of the plant. Apparently, a toxin is carried upward and arrests further activity of the meristematic tissues directly above the lesion.

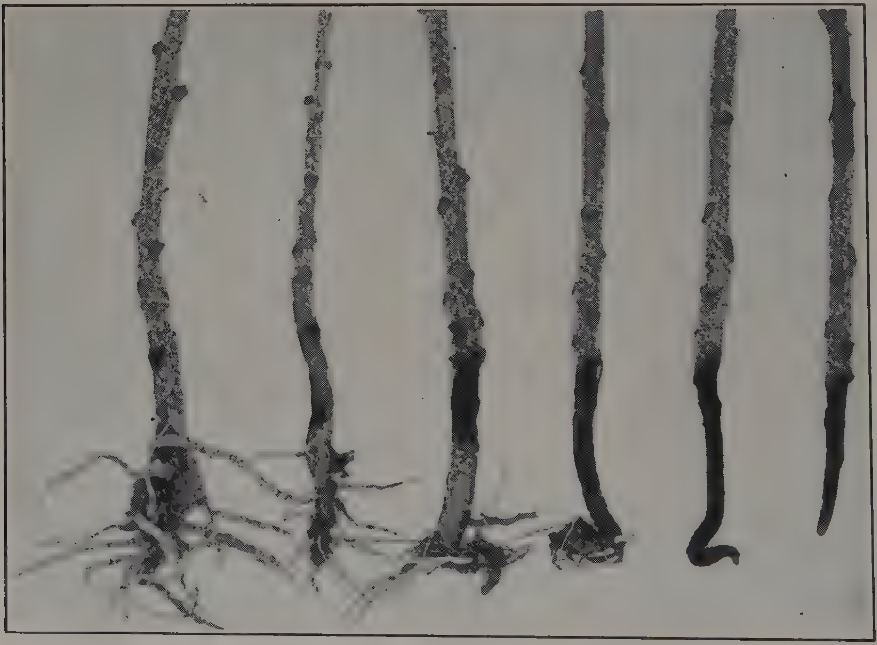

FIG. 2. Progressive stages of Rhizoctonia foot-rot on annual stocks.

ETIOLOGY OF FOOT ROT

Tissue platings have been made from diseased plants of various sizes submitted by growers in many different parts of New York State. Strains of *Fusarium* and *Pythium* were sometimes isolated, but these failed to produce any infection whatever in inoculation experiments. More commonly, strains of *Rhizoctonia* were recovered from affected tissues, and these proved to be pathogenic in all trials. In the pathogenicity tests the organisms were grown in sterilized sand-corn-meal mixture and this was mixed with sterilized soil into which stock seedlings were subsequently transplanted. The seedlings in the Rhizoctonia inoculations were completely destroyed in from 2 to 5 days.

Other tests were conducted in which *Rhizoctonia* was grown in sterilized soil in flasks, and the inoculum thus obtained was subsequently distributed lightly over the surface of flats of sterilized soil in which stocks of various sizes were already established. A light layer of sterilized soil was then sprinkled over the inoculum, every effort being made to avoid mechanical wounding of the plants. By this method it was demonstrated that stocks may remain susceptible to infection until at least 2 months old and 6 to 8 inches tall. Such plants showed initial symptoms of infection within a week and were often completely wilted in less than 2 weeks (Fig. 1). The age or size limit of susceptibility as well as the degree of tolerance might well be affected by any factors, such as light, temperature, soil moisture, etc., which would influence the development of either suscept or pathogen. It has been noted, however, that the disease is severe under all conditions under which glasshouse stocks are commonly grown.

SPREAD OF PATHOGEN THROUGH SOIL

Two 14 × 20 × 3 in. flats of steam-sterilized soil and 4 similar flats of nonsterilized soil were each planted with 30 stock seedlings. Eight days later a tablespoonful of soil-Rhizoctonia inoculum was introduced into the soil at one end of both steamed flats and 2 of the unsteamed flats. Eight weeks after inoculation most of the plants in the rows at the opposite end of all inoculated flats were killed. A total of 58 of the 60 plants in the nonsterilized flats and 54 of the 60 plants in the steamed flats succumbed, whereas no plants in the check flats were affected. This demonstrates the ability of the Rhizoctonia to spread through the soil at least 10 inches per month in all directions from the point of inoculation. Since stocks are in the bench 4 to 5 months before flowering, it follows that all the plants in an area 6½ to 8 feet in diameter about a single point of inoculation would be liable to infection between planting and cropping if progress of the pathogen were not checked.

INOCULATION OF OTHER CRUCIFERS

A single attempt was made to infect several other crucifers with the *Rhizoctonia* from stocks. One flat each of *Alyssum compactum*, *A. maritimum*, *Cheiranthus allioni*, *Iberis umbellata* and cabbage, as well as a check flat of stocks, were inoculated with soil-Rhizoctonia inoculum. The *Iberis* and stock stems were soon girdled at the soil line and the foliage quickly wilted. *Rhizoctonia* was readily recovered from the lesions on the *Iberis* plants in all attempts. The suceptibility of *Iberis* sp. to *Rhizoctonia* has previously been noted by Duggar and Stewart (5) and by Peltier (6). Considerable infection of the leaves and prostrate stems of both species of *Alyssum* developed and a few of the plants were girdled at the soil line. Symptoms were typical of those described by Tilford (7), though he observed no girdling of the stems at the soil line. Typical wire-stem symptoms developed on 3 or 4 of the 30 cabbage seedlings, but no attempt was made to reisolate the pathogen from these plants. No infection had developed in the flat of *Cheiranthus* at the time the experiment was discontinued.

CONTROL RECOMMENDATIONS

Owing to the wide-spread occurrence of parasitic *Rhizoctonias* in field soils and to the serious nature of foot rot, once it becomes established in a planting, it is strongly recommended that all soils be sterilized before planting to stocks. The advisability of this precaution is borne out by the fact that there are few, if any, eastern growers who have not at one time or another incurred serious losses due to foot rot; indeed, many growers experience such losses annually. Complete control has been obtained in our experiments when heavily infested soil was either steam-sterilized or treated with chloropicrin at the rate of 10 cc. per cubic foot of soil. Losses in unsterilized check flats were consistently 100 per cent.

Growers have reported excellent results in checking the spread of foot rot in unsterilized soil by immediately removing infected individuals and drenching the surrounding soil with Cuprocide 54 (cuprous oxide) at the rate of 1 oz. to 2 gal. of water. This treatment failed to give satisfactory control in our tests, however, and resulted in considerable injury to the healthy plants.

SUMMARY

A serious foot rot of stocks (*Matthiola incana*), characterized by girdling of the stem at the soil line and complete wilting of the foliage, is prevalent in eastern cut-flower establishments. Inoculation experiments have proved that foot rot is caused by one or more strains of *Rhizoctonia.* Infection occurs at or just below the soil line, though the entire root system may eventually be involved. Plants up to at least 2 months old are susceptible to infection. The pathogen may spread through the soil at the rate of approximately 10 in. per month. *Alyssum compactum, A. maritimum,* and *Iberis umbellata* were found to be susceptible to *Rhizoctonia* from stocks. Control of foot rot was obtained by sterilizing heavily infested soil with either steam or chloropicrin.

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LITERATURE CITED

1. ANON. [Rhizoctonia stem rot of stocks reported from New Jersey]. U.S.D.A. Pl. Dis. Rptr. Suppl. 86: 87. 1935.
2. ———. [Rhizoctonia root rot of stocks reported from Mississippi]. Ibid. 90: 114. 1935.
3. ———. [Rhizoctonia foot and stem rot of stocks reported from New Jersey and California]. Ibid. 96: 277. 1936.
4. BALFE, ILMA G. An account of sclerote-forming fungi causing diseases in *Matthiola, Primula* and *Delphinium* in Victoria. Proc. Roy. Soc. (Victoria) 47: 369–386. 1935.
5. DUGGAR, B. M., and F. C. STEWART. The sterile fungus *Rhizoctonia* as a cause of plant diseases in America. N. Y. (Geneva) Agr. Exp. Stat. Bull. 186: 1–30. 1901.
6. PELTIER, G. L. Parasitic Rhizoctonias in America. Ill. Agr. Exp. Stat. Bull. 189: 283–390. 1916.
7. TILFORD, P. E. A Rhizoctonia disease of sweet Alyssum. Phytopath. 20: 587–590. 1930.

PHYTOPATHOLOGICAL NOTES

The Growth of Fusarium oxysporum cubense in the Soil.—The commercial banana (Gros Michel) has been ravaged by Panama disease in many of the important banana-growing areas. The great variation in the rate of spread of the organism has been attributed to soil conditions. This study has been carried out to determine the rate of growth of *Fusarium oxysporum* Schl. var. *cubense* (E.F. Sm.) Wr. and Rg. in the soil.

The growth of *Fusarium oxysporum cubense* on the surface of soils of different texture was observed. Three soils were employed: one, a modified greenhouse compost; the next a mixture of equal parts coarse river sand and greenhouse compost; and a third, a clay subsoil. The soil was distributed evenly in long glass tubes, saturated, autoclaved, and inoculated with a single-spore culture. In table 1 the greenhouse compost produced a growth of mycelium that at times grew twice as fast as that on the clay. The total growth for the greenhouse compost was 19.7 cm. in 42 days, or a daily average of 0.47 cm. and the clay 12.4 cm. in 42 days, or a daily average of 0.3 cm. The greenhouse compost averaged 0.38 cm. per day the first 12 days, while during the last 9 days it averaged 0.51 cm. The variation in the clay was less than in the greenhouse compost.

A number of soils was collected for a study of the penetration of the organism into soils of various texture. The soils under consideration may be divided into 2 groups: The first, loam, silt loam, and silty clay loam, tend to hold back the growth of the organism; while the second group, sandy loam, fine sandy loam, and coarse sandy loam favored the growth of the pathogen. The results are presented in table 2. In the first group the average growth for 3 tubes in the 8-day period ranged from 2.46 cm. to 2.96 cm., with a daily average growth of 0.3 cm. to 0.37 cm. In the second group the sandy loams produced in the same period an average growth of 4.03 cm. to 4.2 cm., with a daily average of 0.5 cm. to 0.52 cm. This agrees with Reinking,[1] who, by the plate method, found in soil population around dis-

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[1] Reinking, O. A. Parasitic and other fusaria counted in Costa Rica and Panama soils. Centralbl. f. Bakt. II. Abt. 90: 4–17. 1934.

TABLE 1.—*Average daily growth, in centimeters, of Fusarium oxysporum var. cubense over different periods on different soils. 1. Average for each period. 2. Average for first 33 days. 3. Average for last 30 days. 4. Average for 42 days*

<table>
<tr><th colspan="5">A. Greenhouse compost</th></tr>
<tr><td>1. Average for each period</td><td>0.38</td><td>0.44</td><td>0.60</td><td>0.51</td></tr>
<tr><td>2. Average for first 33 days</td><td colspan="3">0.46</td><td></td></tr>
<tr><td>3. Average for last 30 days</td><td></td><td colspan="3">0.50</td></tr>
<tr><td>4. Average for 42 days</td><td colspan="4">0.47</td></tr>
<tr><th colspan="5">B. Greenhouse compost and river sand</th></tr>
<tr><td>1. Average for each period</td><td>0.33</td><td></td><td></td><td>0.39</td></tr>
<tr><td>2. Average for first 33 days</td><td colspan="3">0.35</td><td></td></tr>
<tr><td>3. Average for last 30 days</td><td></td><td colspan="3">0.37</td></tr>
<tr><td>4. Average for 42 days</td><td colspan="4">0.36</td></tr>
<tr><th colspan="5">C. Clay subsoil</th></tr>
<tr><td>1. Average for each period</td><td>0.27</td><td></td><td></td><td>0.24</td></tr>
<tr><td>2. Average for first 33 days</td><td colspan="3">0.31</td><td></td></tr>
<tr><td>3. Average for last 30 days</td><td></td><td colspan="3">0.30</td></tr>
<tr><td>4. Average for 42 days</td><td colspan="4">0.30</td></tr>
</table>

0 12 days 25 days 33 days 42

eased banana plants that soils of light texture contained more *Fusarium oxysporum* var. *cubense* than did clay soils.

TABLE 2.—*Rate of penetration of Fusarium oxysporum var. cubense in thirteen different soils*

Soil type	Growth in centimeters				
	After 8 days				Daily average
	Tube 1	Tube 2	Tube 3	Average	
Loam	2.5	2.5	3.3	2.7	0.34
Silt loam (A)	2.7	2.5	2.6	2.6	0.32
Silt loam (B)	2.7	3.1	2.7	2.83	0.35
Silt loam (C)	2.8	2.4	2.2	2.46	0.3
Silt loam (D)	2.7	2.9	2.8	2.8	0.35
Silty clay loam (A)	2.8	2.4	2.3	2.5	0.31
Silty clay loam (B)	3.1	3.1	2.7	2.96	0.37
Fine sandy loam	4.4	4.1	3.6	4.03	0.5
Coarse sandy loam (A)	4.5	3.9	4.2	4.2	0.52
Coarse sandy loam (B)	4.3	3.7	4.3	4.1	0.51
Clay subsoil	2.9	2.8	3.7	3.13	0.39
Greenhouse compost	3.8	4.0	4.0	3.93	0.49
River sand	3.9	3.1	3.4	3.46	0.43

The organism was grown in greenhouse compost in 3 different experiments under similar conditions of light and temperature. Although there was considerable variation in daily growth, the organism penetrated the soil 5.0 cm. at about the same rate as it spread on the surface.

The daily average surface spread ranged from 0.24 cm. to 0.60 cm., while the penetration ranged from 0.3 cm. to 0.52 cm.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Highgate P. O., Jamaica, B.W.I.

Infection with Pure Cultures of Clitocybe tabescens.—A root-rot disease of pear and tung trees has been under investigation during the past several years. *Clitocybe tabescens* (Scop.) Bres. has been found constantly associated with the disease, and has been repeatedly isolated from affected trees. Repeated inoculations of pear and tung trees of varying ages with pure cultures of the fungus, made during the period between 1932 and 1937, failed to produce infection. Rhoads[1] also has been unable to obtain infection with pure cultures of the fungus, although he has succeeded in infecting two Australian pine trees by using pieces of infected roots from another tree as inoculum. He does not state what medium he used as his pure-culture inoculum. In our early inoculations the inoculum consisted of pure cultures of the fungus on agar or on autoclaved beanpods. It was noted that this type of inoculum was attacked and disintegrated by various soil-inhabiting saprophytes in a relatively short time (10–15 days), and it was thought that the failure to obtain infection might be due to the destruction of the inoculum before the fungus could invade the root. Even in culture, *Clitocybe* makes a very slow initial growth, a week to 10 days often elapsing before a transfer begins to grow, although growth is fairly rapid after it commences. It was decided, therefore, to find an inoculum medium that would persist in the soil for a long time.

In recent years a method of inoculation has been used that has given more satisfactory results. The fungus is grown on autoclaved thick pieces of tung or pear wood in $\frac{1}{2}$-gal. jars. A spoonful of cornmeal and about 250 cc. of water are added to each jar before autoclaving. The fungus makes a very luxurious growth on this medium, and, in about 4–6 weeks, the wood becomes permeated with mycelium, and rhizomorphs are produced in abundance. This method is similar to that used by Thomas[2] in his studies on *Armillaria mellea.*

On September 28, 1937, 4 old (8-year-old) and 8 young (planted in February, 1937) pear trees, variety Pineapple, were inoculated below ground on one or more of their main roots and at the base of the trunks, using the above described inoculum. The tissue was wounded at the point of inoculation. On February 8, 1938, six additional young trees were similarly inoculated.

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[1] Rhoads, A. S. Clitocybe mushroom rot of citrus trees and other woody plants in Florida. Fla. Agr. Exp. Stat. Ann. Rept. **1932–33**: 120–121; **1933–34**: 76; **1934–35**: 91; **1936–37**: 99; **1937–38**: 116–117.

[2] Thomas, Harold E. Studies on *Armillaria mellea* (Vahl.) Quel.: Infection, parasitism, and host resistance. Jour. Agr. Res. [U.S.] **48**: 187–218. 1934.

On May 17, 1938, one of the young trees inoculated on September 27, 1937, was found wilting and its foliage a pale green. The tree was dug up and examined. The main roots and also the trunk from the crown to about 12 inches above-ground were dead. White plaques of rhizomorphs were present between the wood and the bark and between layers of the bark. The inoculum pieces were still relatively intact (Fig. 1). The fungus was isolated both from the tree and the inoculum.

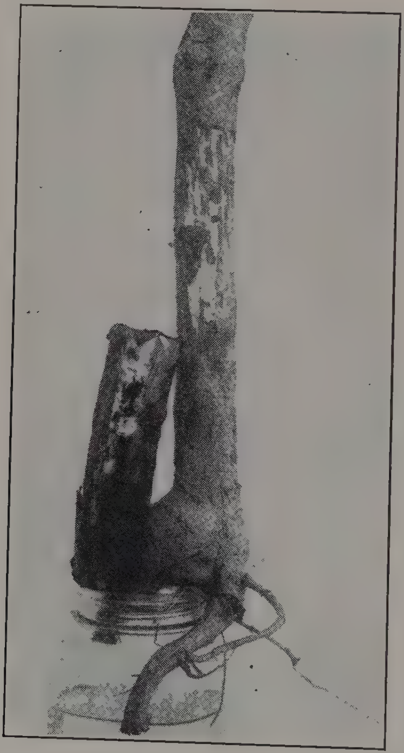

FIG. 1. Young pear tree killed as a result of inoculation with *Clitocybe tabescens* and inoculum piece. Note the white mats of mycelium under the bark of both the tree and the inoculum piece.

On April 16, 1940, one of the 6 trees inoculated on February 8, 1938, was found dying with typical symptoms. In this case, a rhizomorphic strand was found connecting the inoculum piece with the tap root at the base of the trunk. The fungus was again reisolated from both the tree and the inoculum pieces.

Two of the 4 old trees, inoculated on September 28, 1937, were blown down by a windstorm on March 29, 1940. These trees were showing no symptoms on their aboveground parts, but, when dug up and examined, both were found infected. One of the trees had a large lesion, about 8 inches long and more than half way around, on one of its main roots, and 2 other lesions on the trunk just above the junction of the main roots. One of the trunk lesions was about 10 inches long by 4 inches wide and about ½ inch deep. The other tree bore 2 similar lesions on its trunk, but no root lesions. The inoculum pieces were present and intact, except for 1 piece that was riddled by termites, and the fungus was reisolated from them. There is now indication that some of the other inoculated trees are infected, though definite aboveground symptoms are still absent. The underground parts of these trees have not been examined.

This is the first time, as far as the writer is aware, that infection was obtained from pure-culture inoculations with *Clitocybe tabescens.*—A. G. PLAKIDAS, Louisiana Agricultural Experiment Station, University, La.

Algal Leaf and Fruit Spot of Guava.—The alga, *Cephaleuros virescens* Kunze (*C. mycoidea* Karst.), occurs on a wide range of plants in southern Florida. Nineteen species were listed as its hosts in the vicinity of Homestead, Florida, in 1936.[1] Twenty-five additional hosts have been found since then, and a complete list for the region probably would exceed 50 species.

Cephaleuros virescens occurs as a simple epiphyte on the leaves of many of its host plants, where its presence causes little or no damage. On several plants of economic importance, however, the alga may become more or less parasitic and cause injurious effects. For the most part injury attributable to its parasitism is the result of twig or bark infection. Stunting, even death, of small branches may follow severe infection on some hosts, particularly if the plants have been previously weakened by unfavorable growth conditions or other agencies. As a rule the fruits of host plants in southern Florida escape infection. Winston[2] recently described a rather rare fruit blemish on the orange, caused by *C. virescens*, and a similar fruit blemish has been observed infrequently by the writer on unsprayed fruits of the avocado.

The parasitism of *Cephaleurus virescens* to the common guava (*Psidium guajava* L.) is, therefore, of special interest. Twig and bark infections are of little or no consequence on this species in southern Florida, but the leaves and fruits frequently are severely attacked. Some seedlings or varieties are decidedly more susceptible to infection than others. A large-fruit Peruvian variety recently introduced into Florida is especially subject to attack. Partial defoliation may occur and the fruits may be severely blemished unless protective sprays are applied.

Foliar spots vary from mere specks up to 8 mm. in diameter. They may be numerous or few, crowded or scattered, and may occupy any portion of the leaf. They are roughly circular with irregular borders (Fig. 1); reddish-brown to reddish-purple at first, sometimes becoming a rusty brown with reddish margins. Penetration of the leaf blade is complete; affected cells eventually die. The rusty-red fructifications of the alga generally are confined to the lower surface of the spot. They begin to appear about April 1, but are found in greatest quantity from May to August, when they become progressively less evident until, by October, they are difficult to find.

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[1] Ruehle, Geo. D. An epiphytotic of alga spot in South Florida. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 20: 221–222. 1936.

[2] Winston, J. R. Algal fruit spot of orange. Phytopath. 28: 283–286. 1938.

The blemishes produced on the fruit are quite similar to the algal spot on orange fruit, as described by Winston. Individual fruit blemishes are smaller than the foliar spots, seldom exceeding 2 mm. and mostly less than 1 mm. in diameter. They are a dark greenish-brown to almost black, and are slightly raised, with irregular, dendritically lobed margins. Infections apparently may take place at any time up to maturity. Cracks frequently develop in older blemishes as a result of enlargement of the fruit. Penetration of the fruit is confined to several layers of cells beneath the epidermis. Fructification of the alga are seldom seen on the surface of the fruit blemishes.

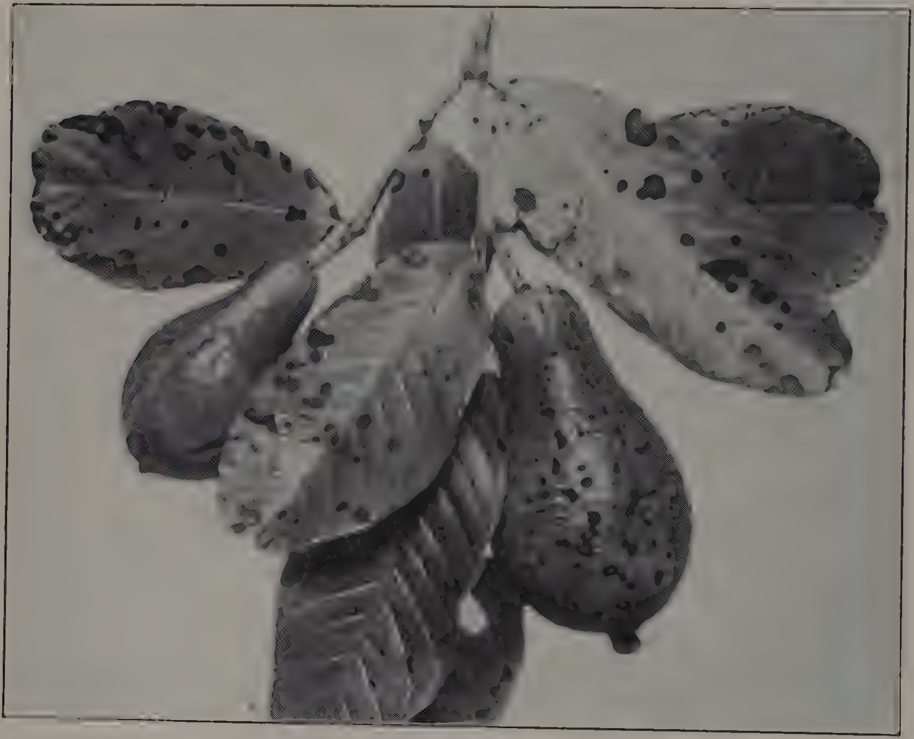

FIG. 1. Leaves and fruits of guava showing spots caused by *Cephaleuros virescens* Kunze.

Infections are more numerous on leaves and fruits developing close to the ground than on those forming at higher levels. Close planting apparently favors the development of the disease.

Spraying with a copper fungicide every 3 to 4 weeks throughout the rainy season has given good control in tests of one season. Either Bordeaux mixture 3-3-100 or its fungicidal equivalent in one of the "neutral" or "fixed" coppers has proved effective. Sulphur sprays applied on the same dates gave little reduction of infection.—GEO. D. RUEHLE, Sub-tropical Experiment Station, Homestead, Florida.

Conidial Production in Culture by Cercospora nicotianae.[1]—Many species of *Cercospora* produce conidia in culture only sparingly or not at all. Hill[2] has reported that he has not observed conidia in single-spore cultures of *Cercospora nicotianae* E. and E. grown on several types of media, including potato-dextrose agar and plain tobacco agar.

In our studies it is desirable to have an abundant supply of spores of *Cercospora nicotianae* to make inoculations. Therefore, we attempted to induce sporulation in culture. Recently a number of isolations of *C. nicotianae* were made from tobacco frogeye spots. Most of these isolates produced conidiophores and conidia on plain tobacco agar.[3]

Between February 5, 1940, and March 14, 1940, 88 insolates were obtained from cured tobacco leaves. After isolation they were transferred to potato-dextrose agar slants, and retransferred March 19, 1940. The subcultures were incubated at laboratory temperature 3 days and stored at 4° C. until May 1, 1940. Spores were not seen.

On May 1, 1940, a new lot of tobacco agar was prepared. Six hundred g. of yellow and green leaves were used, from the lower quarter of Burley plants growing in a greenhouse ground bench. The plants were just beginning to bloom. The leaves were ground in a food chopper, placed in 2 l. of distilled water, and steamed in an autoclave 1 hr. without pressure. Sixty grams of agar was dissolved in a liter of distilled water and added to the leaf decoction. The mixture was strained through cheesecloth and a pad of cotton, then autoclaved. Plates were poured. While the agar was still soft, inoculum from potato-dextrose agar slants, seeded March 19, 1940, was stirred into the agar. The plates were incubated at 27° C. in a chamber without light.

Twenty-seven isolates were tested. By May 3 numerous colonies were visible on all plates. By May 4 most of the cultures were forming conidia. On May 6, conidiophores and conidia were being produced by 26 of the 27 cultures.

An additional group of 61 isolates was tested on tobacco agar plates May 7, 1940. By May 11 most of these were producing spores. By May 14, 60 of the 61 cultures were sporulating. In addition, 2 cultures isolated August 31, 1938, were tested. Both produced spores. One culture, isolated January 16, 1938, did not sporulate.

There was considerable variation in the amount of sporulation among the different isolates; some produced conidiophores and conidia profusely, others less abundantly, some sparingly, and 3 not at all. The spores produced in culture were similar in shape and size to those produced on natural frogeye leaf spots. The conidiophores, however, were much longer and straighter, with geniculations farther apart, than on conidiophores produced on leaves.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.

[2] Hill, A. V. Cercospora leafspot (Frogeye) of tobacco in Queensland, Commonwealth of Australia. Council for Scientific and Industrial Research. Bull. 98. 1936.

[3] Dr. T. C. Ryker suggested to the writers that low carbohydrate media be tried, to produce sporulation.

This difference may have been caused by the high humidity in the Petri dishes. On agar, conidiophores usually were produced singly, directly from prostrate hyphae rather than in groups from sclerotial knots, as they are on leaf tissue. Typical frogeye spots have developed on tobacco leaves atomized with spores produced on the tobacco agar. It was observed that after spores are harvested from a plate by washing the surface of the agar, new spores may be produced within a few days.—STEPHEN DIACHUN AND W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

BOOK REVIEWS

WALKER, JOHN CHARLES. *Diseases of Vegetable Crops,* first revision, 67 pp. (For sale by author, $1.75.) Edwards Brothers, Inc., Ann Arbor, Michigan, 1939.

The revised edition of this useful manual is but little changed in scope and size from the original 1936 edition. The revision is welcomed, however, since some 30 additional diseases are listed, significant new findings are noted, and the literature lists that follow the account of each disease have been reedited and brought down to date.

About the size of the ordinary laboratory notebook, the manual is photolithographed in double-column format from well-prepared typewritten copy. It has a heavy, tough paper cover, and is so bound as to permit it to lie open and flat at any page. The text is confined to one side of the page, leaving the opposite side free for inserting additional notes or references. There are no illustrations.

Under the chief types of garden vegetables grown in the United States, including white (Irish) potato and sweet potato, more than 375 distinct diseases are listed. The major diseases are briefly discussed under the following headings: hosts, history, occurrence, causal organism and its life history, pathological histology, environmental relations, varietal resistance, and control. Pertinent facts are given relating to the production, culture, and marketing of many of the crops. The information about each host and disease is carefully chosen and is formulated in a particularly clear and concise manner. The author has not attempted complete bibliography but has spared others time and effort by selecting with critical judgment some of the more comprehensive and significant contributions on each disease, not overlooking those that contain important literature lists. There is an alphabetical index, arranged according to the common name of the host plant, under which the diseases are listed by common name. Diseases induced by nutritional unbalance, viruses, and insect toxins are included along with those caused by fungi, bacteria, nematodes, etc.

The text has been prepared with care and discrimination by an author who is recognized as an authority on vegetable diseases, having been engaged for many years in active and fruitful research and instruction in this field at the University of Wisconsin, where he has been connected with the State Agricultural Experiment Station and the U. S. Department of Agriculture.

This compact, systematically organized, timesaving reference work will continue to prove useful to advanced students, investigators, college instructors, extension workers and inspectors concerned with vegetable diseases. Many agricultural advisers and teachers of vocational agriculture located in truck-crop-producing areas will doubtless also find the work helpful.—HOWARD P. BARSS, Office of Experiment Stations, U. S. Department of Agriculture, Washington, D. C.

LEACH, J. G. *Insect Transmission of Plant Diseases.* 615 p., 238 fig. $6.00. McGraw-Hill Book Company, Inc. New York and London.

This book probably will become a phytopathological milestone. For a first edition and the first comprehensive treatise on the subject, it is remarkably mature. Enjoyable reading, it should be valuable literature for all pathologists and entomologists, many other biologists, and some laymen. The general implications of most of the experimental data are well thought out and correlated with other phenomena. The author has acquired a viewpoint that suggests interesting lines of research leading from the present meagre data on many insect-pathogen relationships. The reader inclined to research can hardly fail to acquire a similar outlook and be stimulated to reinvestigate some of the problems scarcely disclosed at present.

Leach does not confine himself to a bare outline of the insect transmission of plant diseases. Proceeding from a keen appreciation of the ignorance of the plant pathologist in this field, he introduces and discusses many pertinent subjects that help to elucidate the problem. The discussion of these related topics is one of the most valuable features of the book. The only one of these subjects that seems dispensable is the short discussion on insect-catching plants, which, although interesting, seems rather tenuously related to the main theme.

In the introduction, the author presents a bold and vigorous discussion of the causes for the neglect from which the insect transmission of plant diseases has suffered. Included among these are the early and inevitable domination of plant pathology by mycology, the restricted viewpoint of both the plant pathologist and the entomologist, and the difficulties in the way of cooperation between scientists of the two branches. Among other recommendations, the author suggests the training of some students in the techniques and knowledge necessary for the solution of problems in these insect-pathogen relationships.

The principal sections of the book deal with insect transmission of bacteria, fungi, viruses, and protozoa that cause plant disease. Each of the pathogenic groups is dealt with exhaustively, the better understood cases being presented in detail. Diseases caused by insect toxins, such as psyllid yellows of potato, are treated in a separate group. The anatomy and physiology of insects and plants in relation to the functioning of vectors are dealt with in separate and informative chapters. Feeding and breeding habits and their bearing on transmission receive special consideration. Indeed, many interrelationships of plants and insects are discussed, including the subject of insect pollination. The mutual advantage inherent in insect pollination has a direct and important significance in understanding a distinctive characteristic of the book—the emphasis on mutual benefits of many insect-pathogen combinations. The author points out such beneficial associations throughout the book and devotes a chapter to symbiosis between insects and microorganisms. Mutual benefits not at once apparent have been definitely established in many cases. In other instances, although experimental confirmation is lacking, the author's reasoning should stimulate investigators to test the suggestions of mutual advantage. Although Leach does not point out any cases in which the insect carrier is detrimentally affected, either directly or indirectly, by the service it performs for the pathogen, one would expect such cases to exist. At least, where cultivated plants have been exposed to new insect-pathogen combinations, the insect might be indirectly affected if the pathogen were very destructive to the plant.

Other features of the book are chapters on insect transmission of animal diseases, transmission by mites, nematodes, and other small animals, kinds of inocula transferred, techniques used in insect transmission studies, an appendix summarizing in table form the detailed significant facts about insect transmission of plant diseases, and a complete glossary of the terms employed. The book is liberally supplied with well-chosen illustrations.

There are, naturally, a few errors and omissions of important facts. Among these may be noted the following. The author mentions only Davis, Frobisher, and Lloyd's earlier negative results on multiplication of yellow-fever virus in the mosquito, but, in 1937, Whitman presented convincing evidence that this virus does multiply in its insect carrier. The author fails to consider multiplication in the insect as a possible factor in the incubation period in vectors. It has been pointed out to the reviewer that the flea is now known to be a vector of endemic typhus in rats so that the louse, mentioned as the sole vector, must be demoted from this exclusive position. In the section on agents of virus dissemination, the possibility of transmission by parasitic higher plants is not mentioned, although such transmission has recently been demonstrated by Bennett. Such faults are trivial when considered in relation to the vast amount of information that has been compressed and correlated in this publication.—L. M. BLACK, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

GENAUX, CHARLES M. and JOHN G. KUENZEL. *Defects which Reduce Quality and Yield of Oak-hickory Stands in Southeastern Iowa.* Agr. Exp. Stat. Iowa State College of Agriculture, and Mechanic Arts. Research Bull. 269: 409–444, illus., 1939.

This bulletin focuses attention on timber defects in a region that has received scant attention in this regard, and where the small proportion of woodland to agricultural land puts a premium on wood values. The study was concentrated in young stands in the southeastern corner of Iowa, where all but a small percentage of the trees were under 10 inches in diameter. Over 700 trees on 12 sample plots were dissected.

The defects encountered were classified as decay from fire scars, butt rot, branch-stub defects, insect defects, and miscellaneous. The miscellaneous defects included such as were caused from logging, frost cracks, ice, etc. Decay and other defective wood were measured in cubic feet and expressed both as cull volume and as percentage of total volume defective. The percentage defect was computed separately, by defect type, for each of 4 diameter classes. The defect percentages ranged from 5.2 to 8.5 among the diameter classes, with no apparent trend, except in the case of white oak, where the defect percentage decreased consistently with diameter.

Since only 8 per cent of the trees had fire scars, healed or open, the cull from this source was small. There was also very little cull, (about 1 per cent) from decay that entered sprouts from the parent stump, although there had been quite a fair percentage of such transmissions cases. There was a small percentage of cull from branch stubs. The bulk of the defects were from insects and miscellaneous sources.

A higher proportion of the defects were not visible than were visible in the standing tree. It is pointed out that this is a limitation in estimating defects. Decay had spread considerably farther behind open branch stubs than closed stubs of similar size.

Of the 17 fungi isolated from decaying trees *Stereum gausapatum* was by far the most common, being responsible for the decay in 23 out of 26 butt-rot isolations, 1 out of 17 branch-stub isolations, and 2 out of 12 other isolations. *Poria andersonii* was recovered from 4 of the 17 branch-stub-decay isolations. No other fungus was outstanding in frequency of occurrence.

Primary wood borer activity, in sound wood, was very common in the oaks and hickories. Other insects were associated with wounds and decayed wood. A list of insects collected is given.

Since the trees studied were largely below saw-timber size, cubic measure had to be used. In scaling defects in saw-log trees, cubic measure will always give a much lower percentage of cull than board-foot measure, since it does not take into account the waste in sound wood in cutting out defects. The unavoidable use of cubic measure in the present case tends to make the losses seem much smaller than they would be were the same defects present in larger trees.

The bulletin gives a thorough introduction to the subject of the misuse of forest land in southeastern Iowa leading to defective timber. The data are well presented and the bulletin is adequately illustrated.—GEORGE H. HEPTING, Division of Forest Pathology, Bureau of Plant Industry, U. S. Dept. of Agriculture, Asheville, N. C.

PEROTTI, RENATA. *Biologia Vegetale Applicata all' Agricoltura. III. Micologia—Malattia Parassitarie. (Plant Biology Applied to Agriculture. III. Mycology—Parasitic Diseases).* Rosenburg and Sellier. (Turin) 2nd Ed. 1940.

The work of 1190 pages here reviewed is one of a set of five volumes. The remaining four cover nonparasitic diseases, bacteriology, soil bacteriology, and microbiology in the industries, and technique. A preliminary section of the chapters dealing with parasitic diseases includes the taxonomy, morphology, and physiology of the organisms discussed in the chapter. The individual diseases are discussed in the following order: history, symptoms, etiology, host-parasite relationship, conditions favoring infection, and control.

A general review of the nature of parasitic diseases is presented in the first chapter. Neoplastic bacterial diseases of olive, grape, conifers, oleander, punice, peach, and potato are covered in Chapter 2. Chapter 3 reviews crown gall (including E. F. Smith's work), and other gall diseases, as well as the morphology of galls. The various theories advanced as to the cause of hypoplasias, hyperplasias, and hypertrophies, as well as the pathological anatomy involved, are discussed in the fourth chapter. The bacteria causing necroses are treated in Chapter 5, the principal references cited being the publications of E. F. Smith *et al.* A 22-page key of phytopathogenic bacteria, which follows the old classification, is also included in this chapter.

Viroses are discussed in Chapter 6 under the title "Diseases caused by ultramicrobes." The American work up to 1937 (Stanley *et al.*) is included as well as the earlier work on peach yellows and rosette. Chapter 7 relates to diseases caused by myxomycetes. Club root of crucifers (*Plasmodiophora brassicae*) and powdery scab of potatoes (*Spongospora subterranea*) are included in this group. Chapter 8 treats with the morphology, cytology, and physiology of fungi, and Chapter 9 discusses reproduction and taxonomy of fungi.

Chapters 10 to 28 deal with diseases caused by specific groups of fungi, commencing with the lower forms, through the ascomycetes and basidiomycetes and finally to the fungi imperfecti. Considering the large number of fungi and diseases covered, these chapters appear to be well-handled. Chapter 29 includes diseases caused by algae, lichens, and dodder. The last chapter reviews the problem of immunity in plants in considerable detail. In addition, general control measures also are discussed, with particular attention given to the use of a number of proprietary fungicides.

Dr. Perotti has combined a good course in mycology with problems of interest to the plant pathologist. Considering its wide scope, the book is, in most instances, a comprehensive compilation of the more important papers published in the field of mycology and plant pathology. A few very obvious omissions occasionally occur, however, despite its rather complete coverage. For example, in the treatment of the Dutch elm disease, not a single American publication is cited. Considering the large number of worthy publications by American investigators, this oversight is inexcusable. The nomenclature does not conform in many instances to that in common current use among American investigators. There should, however, be no difficulty in determining the specific disease under discussion.

For obvious reasons Italian investigators are cited most often. Numerous references are inserted as footnotes throughout the book, and more than 400 drawings and photographs, including an excellent color plate of 4 common cereal rusts, all help to enhance the value of the book. Three indices (authors,' geographical, and subject matter) also help to make the book more usable.

Its preparation doubtless involved years of reading and thought; and, despite the few criticisms mentioned above, I believe Dr. Perotti's present volume is the best and most complete Italian work combining mycology and plant pathology, that has yet been published. Unfortunately, the lack of a good Italian-English botanical dictionary will limit its use among American mycologists and plant pathologists.—P. P. PIRONE, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

PATHOGENIC AND CULTURAL VARIATION AMONG SINGLE-SPORE ISOLATES FROM STRAINS OF THE TOMATO-WILT FUSARIUM

FREDERICK L. WELLMAN AND DOROTHY J. BLAISDELL

(Accepted for publication July 2, 1940)

Fusarium bulbigenum var. *lycopersici* (Brushi) Wr. and R. is a vascular parasite, the cause of the common, widely distributed, and important Fusarium wilt of tomato. In connection with the breeding of wilt-resistant tomato varieties, detailed studies by the writers (10, 11) on the variability of the causal organism revealed a wide range of cultural and pathogenic characters among isolates that were then grouped into 5 different classes.

The work reported in this paper[1] deals with: 1. Range of cultural variability and relative stability in strains.[2] 2. Direction of change in the cultural character and in the pathogenicity of variable strains as revealed by their monoconidial isolates. 3. Isolation of strains markedly different from that of their original form, through use of variants found among single-spore isolates. 4. The possible existence of a basic culture type, or class, from which all other strain classes might arise.

These studies corroborate certain phases of the findings of others on other fusaria, such as those of Leonian (5), Eide (2), Ullstrup (8), and Snyder (7), and further suggest some explanations of difficulties experienced in tests of strain pathogenicity in and host resistance to the tomato-wilt Fusarium. In the instance of Edgerton and Moreland (1) appressed darker-colored *Fusarium* isolates from wilted tomato plants were discarded as atypical for the causal organism. It is possible that in studies on other fusaria, both in mycological and pathological connections, variants may have been treated as contaminants.

The results of studies at this laboratory on the variability of the tomato *Fusarium* have been of very practical value. This may be seen in recent work just completed by Porte and Wellman (6) in the breeding and selecting of tomato varieties that are more highly resistant to wilt than tolerant varieties, like Marglobe and Rutgers. Other activity along these lines is still in progress.

METHODS AND MATERIALS

The strains designated in the present paper are the same as those described in the previous report (11). Having already been studied as to cultural and pathogenic characters, they were selected as particularly suitable parent material for studies in variability. In some instances certain single-spore isolates from "parent" cultures were used as sources of further extensive single-spore studies. The cultures from single-spore isolates from a "parent" culture were considered as the "first generation." Cultures from single spores isolated from "first-generation" cultures were considered as the "second generation," and so on. Under ordinary conditions it required about 2 weeks to isolate and grow each culture generation sufficiently to be able to classify it dependably. Sometimes a slightly longer period was required, especially with the cultures that produced the larger amounts of aerial mycelium.

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[1] The writers wish to acknowledge the assistance of E. C. Tatman on statistical analyses.

[2] The word "strain" refers to single-spore isolates from selections of fungus material. These were called "selections" in a recently published paper (11) although referred to as "strains" in a prior abstract (10). The latter term is used for convenience and does not imply basic and invariable specialization, similar, for example, to the physiologic strains in rust fungi.

All culturing was done on 3 media described elsewhere (11), potato-dextrose, Tochinai, and Leonian agars.[3] Growth was most rapid and could be best classified on the potato and Leonian agars. All inocula for testing pathogenic effects were grown in Tochinai liquid in a standard manner (9). Single-spore isolations were made 1. through the use of a micromanipulator, and 2. by spreading spore dilutions on hardened agar plates and transferring them to other agar plates when single, newly germinated spores could be selected with certainty. In a few cases 2 to 4 single-spore isolates were transferred to a single agar plate to determine culture classes, but generally only one isolate was grown on a plate. No reisolations of any sort were ever made from plates on which more than one colony was growing.

Cultures were classified according to the macroscopic appearance of the colonies and in compliance with criteria already discussed in detail (11). Briefly described, these culture classes or types (Fig. 1) are: Fully *raised* (R), abundant and fine, woolly, light-color, aerial growth with reticulate surface, mat on agar surface easily cut; *raised sclerotial* (RS), light-color, fine woolly to slightly coarse, cottony, aerial growth with irregular tufts and mycelial clumps over the surface, mat on agar surface easily cut; *intermediate raised* (IR), whitish to darker colored, coarse, cottony, aerial growth with occasional irregular strands and a somewhat water-soaked appearance, colored mat on agar surface not easily cut; *intermediate appressed* (IA), whitish to dark-color, short and scanty, coarse aerial mycelium with large water-soaked areas, dark mat on surface of agar, tough; and completely *appressed* (A), very little or no aerial growth, mat on agar surface dark-color and slimy in appearance, tough when cut. Colors, of course, varied somewhat in intensity, depending on media used and other conditions.

Relative pathogenicites of cultures were tested under controlled conditions on tomato seedlings by a standard technique (9) that included numerical evaluation of the plant's response to the pathogen. These evaluations were: 0, signifying no apparent disease in the plant; 1, 2, and 3, increasing amounts of discolored vasculars from tip of tap root up into main body of root region, hypocotyl, and to cotyledonary node; 4, 5, and 6, extension of disease from cotyledonary node up into base of plant, involving successive older leaves; 7, 8, and 9, further severity of wilt, developing through main part of plant up to almost complete wilting; 10 completely wilted, all leaves involved and vascular elements discolored but with stem turgid; and 15, collapse of central stem following complete wilting.

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[3] In these studies 12 g. of agar were added per liter of liquid solution instead of 20 g. The softer agar gave better differentiation of cultural classes.

CULTURAL VARIABILITY OF MONOSPORIC ISOLATES

That variability in the tomato *Fusarium* is common has been noted by a number of investigators, e.g., White (12), Haymaker (3), and Leonian (4). Their observations have been, in the main, corroborated in work by the writers (10, 11) in which the marked instability of the causal organism was

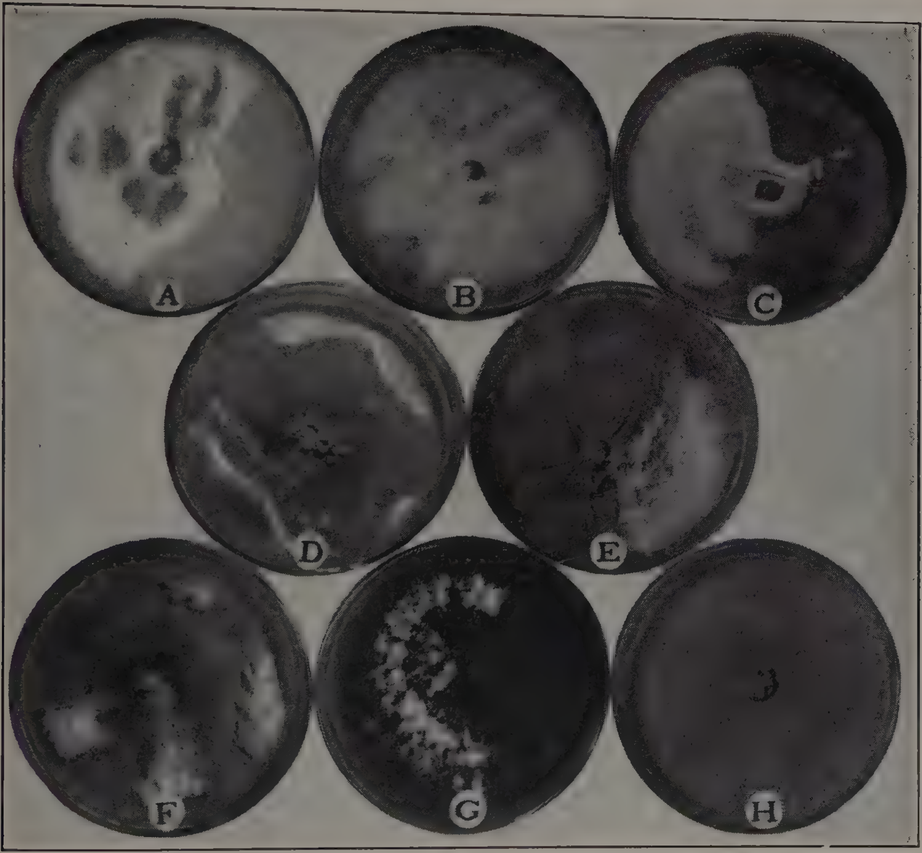

FIG. 1. Examples of cultures of tomato-wilt Fusarium with areas from which single spores were isolated. (Potato-dextrose agar used, cultures 2 weeks old, in Petri dishes 9 cm. in diameter.) Letters designate culture classes as in text under ''Methods and Materials.'' Portions of aerial mycelium typical for growth classes are, in dish C (R), dish B (RS), dish E (IR), dish G (IA). Growth in dish H is typically (A). A. Typical RS culture with sunken areas about center, from which saltating IR cultures were obtained. B. Typical RS; note sclerotia, indistinct sectors from which were obtained RS, IR, IA, and A. C. Unstable R, over half A. This parent produced R, RS, IR, IA, and A. D. varying isolate from dish B. Spores from cultures like this produced IR, IA, and A. E. Culture originally IR with a large, markedly A sector from which monosporic isolates were all A. F. Originally an IR culture with A sectors occurring and becoming predominant. Spores from aerial mycelium produced IR and IA cultures, from A sectors all produced A growth. G. Sector of A type in IA. Spores from aerial mycelium produced IA and A, while from A sector only A developed. H. Typical A isolate with different colored streaks but all spores therefrom produced A growth.

pointed out. The fact was noted that certain selections or strains were relatively free from such irregularities as sectors or saltations in culture, whereas such effects were quite common in other strains. The least variation occurred in the A- and in some of the R-type strains. Culture classes IA, IR, RS, and certain R types produced saltations more or less regularly.

In a few of the isolation studies reported below, obviously unstable portions of fungus growth were avoided as spore sources; but, in most cases, advantage was taken of every evidence of differences arising in cultures to secure possible variants. Differentiating areas arising in cultures were generally easily seen (Fig. 1). No attempts were made to randomize the points from which single spores were selected for culturing purposes, and in the interpretation of the data these facts must be kept in mind. The single-spore cultures were incubated at room temperatures (20° to 24° C.) and culture types determined about a week after they had attained a diameter of 4 to 6 mm. Upon the appearance of possible R characters from RS parents or possible RS characters from R parents, the cultures were kept 2 or 3 weeks longer to be sure of results, since, at times, classification in this regard is difficult on younger cultures.

In this work 2,031 single-spore isolates were grown from 18 parent strains, all of which had originated from monoconidial isolates in connection with previous studies (11). In a few parent strains certain gross transfers produced growth seemingly different from the original. These transfers were handled as separate isolates of the parent strain, and their single-spore progenies were grown for comparison with others from the original parent. Gross transfers were used as original sources of single-spore isolates from 51 parental colonies.

RANGE OF VARIABILITY

Data showing the range of cultural variability are represented in table 1. Single-spore isolates from fully raised, R, parents that had never produced saltants in culture over a long period of time and were known to be consistently high in pathogenicity were all R in character. Likewise, single-spore isolates from completely appressed, A, parents that produced no saltants in culture and were uniformly low in pathogenicity were found to be always A in cultural character. In contrast to the cultures from these two parental types were all the other parent types that were known to be variable in pathogenicity and growth characters. In these and later trials (Tables 1 and 2) the definitely variable R parents were the only class from which isolates were secured exhibiting the whole range of culture classes, namely, the R, RS, IR, IA, and A types. The RS parent strains that were seemingly the most variable both culturally and pathogenically, produced single-spore isolates of RS, IR, IA, and A types but not R. Intermediate parent types, IR and IA, were the source of isolates that were nearly as variable as those from the RS parents. The greatest range of variation occurred among isolates from parent cultures that had abundant aerial growth on artificial media and were generally characterized by presence of saltations. Unstable parents with little aerial growth gave rise to isolates of comparatively less variability.

TABLE 1.—*Range of variability of culture classes found in single-spore isolates from various known parent colonies of Fusarium bulbigenum var. lycopersici* (*one generation studied in each case*)

Parent colony, class and number	Class of culture furnishing spores	Distribution of single-spore isolates under five culture classes[a]:				
		R	RS	IR	IA	A
R5–1	R	24	0	0	0	0
R5–5	R	11	0	0	0	0
R8–1	R	24	0	0	0	0
R17–1	IR[b]	1	0	3	4	0
R17–2	R	30	1	1	0	0
R21–1	IR[b]	14	0	6	4	0
R21–4	R	22	0	0	0	0
RS2–1	RS	0	1	1	4	5
RS2–2	IR[b]	0	5	1	0	2
RS7–1	RS	0	1	6	3	2
RS7–2	RS	0	18	10	6	2
RS7–3	RS	0	7	0	3	2
RS7–5	RS	0	5	45	0	0
RS9–2	IR[b]	0	3	12	2	2
RS12–1	RS	0	25	0	0	0
RS12–2	A	0	0	0	0	7
RS12–3	RS	0	37	6	10	3
RS16–1	IA[b]	0	7	3	3	1
RS16–2	RS	0	18	1	0	3
RS26–1	RS	0	9	10	5	0
RS29–1	RS	0	7	2	0	0
IR10–1	IR	0	0	10	0	0
IR13–1	IR[b]	0	0	11	8	5
IR23–1	IR	0	0	6	2	2
IA3–1	IA	0	0	2[c]	23	16
A1–1	A	0	0	0	0	10
A1–2	A	0	0	0	0	30
A6–1	A	0	0	0	0	22
A6–3	A	0	0	0	1[c]	20
A15–1	A	0	0	0	0	26
A15–3	A	0	0	0	0	29

[a] Definition of classes in text under "Methods and materials."

[b] Portion of culture used for source of spores was a sector. Purification of source not attempted; occasional mixtures of spores from adjacent culture material was expected and evidently occurred.

[c] Cases that appeared to have more aerial mycelium than characterized the cultures from which the spores were isolated. It is believed no mixtures were involved.

DIRECTION OF VARIABILITY

Studies were made to discover any specific directions in which variability occurred among "progenies" of the parent cultures under existing experimental conditions. The results from 20 parent cultures are shown in table 2, and from 5 of these parents in figure 2. It will be seen in tables 1 and 2 that isolates from R5 and R8 were of both R and RS classes, whereas isolates from R17 and R21 gave R, RS, IR, IA, and A characters of growth. It is believed these observations are of consequence, since they indicate that R classes of isolates are the most truly representative of the pathogen. Their importance is pointed out below in the discussion. In the isolates from

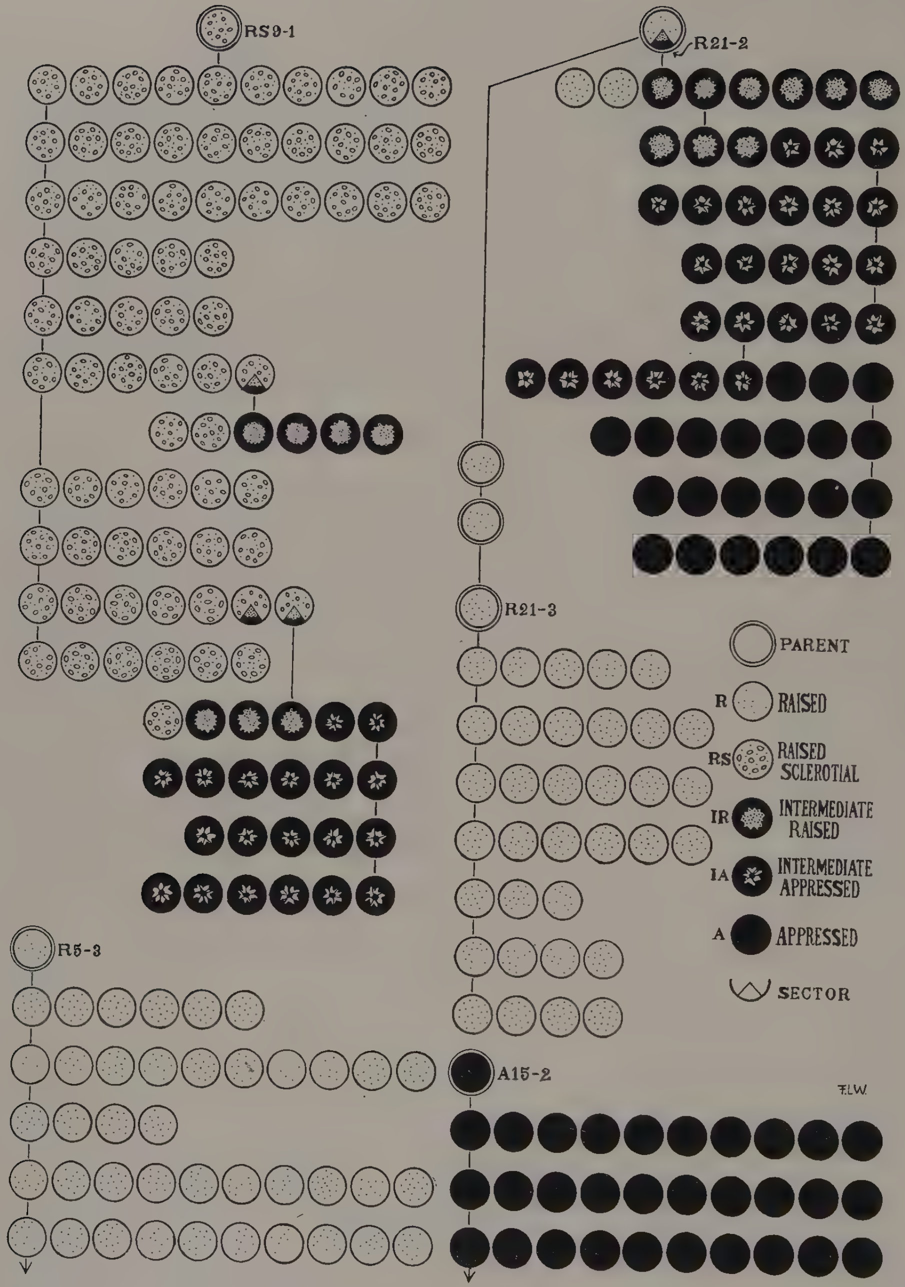
RS9-1
R21-2
R21-3
R5-3
A15-2
PARENT
R RAISED
RS RAISED SCLEROTIAL
IR INTERMEDIATE RAISED
IA INTERMEDIATE APPRESSED
A APPRESSED
SECTOR
F.L.W.

FIG. 2. Results of single-spore isolations showing direction of cultural variation through study of progenies in certain strains of *Fusarium bulbigenum* var. *lycopersici.* (Table 2.) From culture RS9–1, with abundant sclerotia-bearing, creamy-white, aerial mycelium (RS), apparently pure cultures of intermediate appressed (IA) type, dark-brown, slimy growth with scanty aerial, sclerotia-free mycelium, were obtained at the end of 12 generations of monoconidial isolations. A sector of R21 was used, R21–2, that had coarse, cottony growth (IR), while the parent mat was a white, woolly R culture, and from it seemingly pure, completely appressed (A), slimy, dark reddish-purple cultures with no aerial mycelium were obtained after 9 generations. From a nonsectoring part of R21 a transfer was made and purified and from it, R21–3, throughout 7 generations of single-spore cultures all were R. Progenies of stable A15 and R5 cultures were A and R in type, respectively, after several generations.

TABLE 2.—*Direction of variability towards either raised or appressed culture classes, as found in single-spore isolates from certain parent colonies of Fusarium bulbigenum var. lycopersici (from 3 to 13 generations studied in each case)*

Parent colony, class and number	Class of culture furnishing spores	Generation of isolates	Distribution of single-spore isolates under five cultural classes[a]:				
			R	RS	IR	IA	A
R5–4	R	1st	9	0	0	0	0
	R	2nd	5	0	0	0	0
	R	3rd	5	0	0	0	0
	R	4th	7	1	0	0	0
	R	5th	9	0	0	0	0
	R	6th	6	16	0	0	0
R17–3	IR[b]	1st	6	0	2	0	0
	R	2nd	12	0	6	3	0
	IR	3rd	2	0	4	2	0
	IR	4th	0	0	22	4	0
	IR	5th	0	0	20	3	0
	IR	6th	0	0	12	0	0
RS7–4	RS	1st	0	1	0	0	0
	RS	2nd	0	11	1	0	1
	IR[b]	3rd	0	1	4	2	0
	IR	4th	0	0	15	11	3
	IR	5th	0	0	8	4	0
RS16–4	RS	1st	0	4	0	0	0
	RS	2nd	0	4	0	2	3
	A	3rd	0	0	0	0	12
	A	4th	0	0	0	0	32
	A	5th	0	0	0	0	44
	A	6th	0	0	0	0	16
	A	7th	0	0	0	0	4
	A	8th	0	0	0	0	24
IR13–2	IR	1st	0	0	4	0	0
	IR	2nd	0	0	10	2	0
	IR	3rd	0	0	10	7	4
	IR	4th	0	0	20	11	6
	IR	5th	0	0	16	9	3
IA3–2	IA	1st	0	0	0	20	0
	IA	2nd	0	0	0	1	7
	A	3rd	0	0	0	0	16
	A	4th	0	0	0	0	24
A6–2	A	1st	0	0	0	0	24
	A	2nd	0	0	0	0	24
	A	3rd	0	0	0	0	24
R5–2	All R	Total of 3	30	0	0	0	0
R5–3[c]	ʻʻ R	ʻʻ ʻʻ 7	120	0	0	0	0
R21–2[c]	IR, IA, A	ʻʻ ʻʻ 9	2	0	9	25	20
R21–3[c]	All R	ʻʻ ʻʻ 7	34	0	0	0	0
RS2–3	RS, IR, A	ʻʻ ʻʻ 4	0	7	9	0	10
RS9–1[c]	RS, IR, IA	ʻʻ ʻʻ 13	0	74	7	19	6
RS9–3	All RS	ʻʻ ʻʻ 3	0	23	11	0	0
RS9–4	ʻʻ RS	ʻʻ ʻʻ 3	0	34	0	0	0
RS16–3	ʻʻ RS	ʻʻ ʻʻ 5	0	31	7	6	2
IR13–3	All IR	ʻʻ ʻʻ 4	0	0	22	2	0
IA3–3	ʻʻ IA	ʻʻ ʻʻ 5	0	0	0	46	9
IA3–4	IA, A	ʻʻ ʻʻ 7	0	0	0	33	10
A15–2[c]	All A	ʻʻ ʻʻ 6	0	0	0	0	112

[a] Definition of classes in text under ʻʻMethods and materials.ʼʼ

[b] Portion of culture used for source of spores was a sector. Purification of source not attempted; occasional mixtures of spores from adjacent culture material was expected and evidently occurred.

[c] These data represented diagrammatically in figure 2.

parents A15, A6, and A1, all, with one exception, were apparently A in character.

In the first generation of isolates from RS16–4 (Table 2), all appeared to be typically light-color RS in character. After ageing a few weeks 1 of the 4 developed a narrow, dark sector with coarse aerial mycelium and no sclerotia. Very few spores were found, but single-spore isolates were obtained from this sector 3 of which were typically RS, 1 was an RS form that had excessively coarse tufts of mycelium among the sclerotia-bearing aerial growth, 2 were IA, and 3 were A. Monosporic isolates were made from one of these A cultures and from these, A types were secured in the third generation. From then on, through 6 generations, only cultures of A type were consistently secured from spores from the A isolates, and it was believed in the end that a reasonably constant A type of culture had been selected from a parent originally RS in character. In the case of RS16–3, which was carried at the same time, the monosporic isolates produced not only A but IA, IR, and RS types of cultures.

In figure 2 are presented diagrammatically the results on studies of parent cultures R21–2 and R21–3 that had both come originally from R21, a typical unstable R type. Reisolations were carried 9 generations in one line and 7 generations in another, to a total of 98 monoconidial isolates. At the close of these 2 series a stable A type of isolate apparently had been derived from original material from a sector IR in appearance occurring on one side of an otherwise R parent culture. It is notable that reisolations from material obtained from the opposite, and, apparently, more stable side of the mat (Fig. 2) produced nothing but R isolates throughout 7 subsequent generations of reisolation.

Results of monosporic reisolations from 4 parent cultures of RS9–1 were slightly more complex. A total of 184 single-spore isolates were studied. From 1 apparent saltant culture, RS9–2 (Table 1), 19 monosporic isolates were obtained for only one generation, and of these, 3 were RS (probably contaminants from the RS mycelium in which the IR saltant occurred), 13 were close in type to that of the IR type of sector whence the spores came, 2 were IA, and 2 were A in type.

In the case of RS9–1 (Fig. 2 and Table 2), one parent mat was characteristically of RS type after being in culture on agar for over a month and then going through 5 generations of monosporic reisolations. A total of 40 monosporic cultures, all RS, were produced that remained typically RS without evident saltations through 6 weeks' undisturbed retention in culture. One of these RS isolates was used as the source of six 6th-generation cultures which at first appeared RS in character without evident saltations. Later, 1 of the 6 isolates produced a saltant sector IR in character and the 5 monosporic isolations from it (7th generation) produced cultures, both IR and RS in character. A 6th-generation culture that had not sectored was used to carry on the RS character 2 more generations, and 12 cultures of RS character only were produced. In the 9th generation, however, 2 out of 7 isolates had IR type sectors and monosporic isolates obtained from one of these sectors (10th generation) were of the IA, IR, and RS types. One of these IA isolates was held for 3 weeks before monosporic reisolations were again made and for the subsequent 3 generations all isolates from this line were IA in character. It is to be noted that in this case the IA type was the "lowest" type obtainable after manipulation for 13 generations; yet, in another instance from the same strain (RS9–2, Table 1), A types were secured in the first monosporic isolations from a variant portion of one parent culture.

The isolations from A5–3 and A15–2 propagated true to type.

Isolations from the intermediate, IA and IR, types were not so extensive as with the RS cultures, since it was found that range of variation was not so great. However, (Tables 1 and 2) the monosporic isolates from IR and IA cultural classes showed variation and were easily divisible into intermediate and appressed types.

From the above studies it appears to be clear that, in general, the character of growth of single-spore isolates, when it differed from the parental type from which the spores were obtained, was in the direction of less aerial growth, that is "downward," toward the lower, less pathogenic,[4] or appressed (A) types. It is well to emphasize here, however, that variation toward less aerial growth does not always occur, because (Tables 1 and 2) in 10 out of 146 series of spore isolations some of the isolates were "higher" in culture class than the sector whence they were obtained. In these there is evidence that they involved mixture of spores secured from mycelia adjacent to sectoring portions of the mats, and the results are so interpreted. In over 100 cases cultural techniques were especially employed to obviate chance mixture of classes, and in all these series all single-spore isolates were the same class as or "lower" than the culture of origin. However, in one of these series from purified cultures 2 IR cultures occurred among 42 single-spore isolates from a carefully purified IA parent; in another case, one IA culture appeared among 21 monosporic isolates from a known A parent. In both these cases the remaining isolates were of the same class as their parents or lower.

Spore Type and Variability

In the tomato-wilt strains of *Fusarium* used in these studies, 3 types of spores were commonly found, the single-cell microspores, which are slightly elongated and tend to be kidney-shape; the two- to six-cell macrospores that are long and sickle-shape (no attempts were made to keep detailed record of numbers of cells per conidium in the macrospore studies); and the single-cell, rounded, rather thick-walled, hyphal chlamydospores. Chlamydospores that occur in conidia were not found for use in these studies. The hyphal chlamydospore germinates slowly in comparison with microspores and macrospores, and is borne both between cells and at the tips of hyphae, while

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[4] Relative pathogenicity of monosporic isolates is indicated below under the section "Pathogenic variability of monosporic isolates."

TABLE 3.—*Results of single-spore isolations of microspores, macrospores, and chlamydospores from the same parent cultures of the common tomato-wilt Fusarium, with respect to correlation of cultural classes secured from the three spore types*

Parent source class and number	Spore type used and number and class[a] of spore isolations secured							
	Microspores		Macrospores		Chlamydospores[b]		Random isolates[c]	
	Spores used	Classes	Spores used	Classes	Spores used	Classes	Spores used	Classes
R5–3	24	R	......	....	10	R	120	R
R21–3	......	....	20	R	......	....	34	R
RS7–2	18	RS, IR, IA, A	12	RS, IR, IA	9	RS, IR, IA	36	RS, IR, IA, A
RS7–4	14	RS, IR, IA, A	20	RS, IR, IA, A	5	RS, IA	61	RS, IR, IA, A
RS16–2	9	RS, A	......	....	......	....	56	RS, IR, A
RS16–4	17	RS, IR, A	......	....	......	....	145	RS, IA, A
RS12–1	......	....	10	RS	......	....	25	RS
RS12–3	......	....	12	RS, IA, A	......	....	56	RS, IR, IA, A
IA3–2	32	IA, A	......	....	......	....	68	IA, A
A6–1	......	....	8	A	......	....	22	A
A6–2	......	....	20	A	......	....	72	A
A15–2	22	A	......	....	10	A	112	A

[a] Definition of classes in text under "Methods and materials."

[b] All chlamydospores from hyphae.

[c] Includes either microspores or macrospores, or mixtures of these.

the other spores occur on sporophores. Considering these and other possible differences, the question naturally arose as to whether the type of spore might possibly have some effect on the character of cultures from monosporic isolates.

Table 3 shows that each kind of spore produced essentially the same range of cultural classes as did isolations of conidia handled in random manner, nonidentified as to type from the respective parent cultures. There are minute discrepancies in a few cases in which a slightly larger range was noted for the isolates from the random isolation of mixed microspores and

TABLE 4.—*Results on pathogenicity studies of differing strains of Fusarium bulbigenum var. lycopersici on Bonny Best and Marglobe tomatoes, comparing data from parent cultures with the single-spore isolates obtained from these parents*

Test host	Fusarium strain[a] class and number	Cultures used		Pathogenicity evaluations			
		Plants inoculated		Parent culture		All progenies	
		Parents	Progenies	Average index[b]	C.V.[c]	Average index[b]	C.V.[c]
Bonny Best	R5	1/10[d]	21/210[d]	13.4 ± 2.59	19.33	11.9 ± 2.99	25.13
	R17	1/10	29/290	9.4 ± 2.41	25.64	5.7 ± 2.11	37.02
	Mean	......	......	11.4 ± 2.44	21.40	8.8 ± 1.99	22.60
	RS16	1/10	14/140	10.2 ± 3.55	34.80	10.1 ± 4.09	40.50
	RS7	2/20	36/410	10.2 ± 3.03	29.77	8.4 ± 3.36	40.00
	RS12	2/20	32/320	6.8 ± 1.87	27.50	7.6 ± 3.77	49.61
	Mean	......	......	9.1 ± 2.93	32.20	8.7 ± 2.03	23.33
	IR26	1/10	24/240	10.3 ± 2.54	24.66	9.0 ± 2.48	27.56
	IR13	1/10	24/240	9.3 ± 1.06	11.40	7.2 ± 1.51	20.97
	IA3	1/10	39/390	8.1 ± 2.68	33.09	8.5 ± 3.32	39.06
	Mean	......	......	9.2 ± 2.14	23.26	8.2 ± 1.80	21.95
	A15	2/20	23/230	1.6 ± .96	60.00	1.0 ± 1.16	116.00
	A1	1/10	10/100	5.1 ± 1.66	32.00	5.4 ± 1.71	31.67
	A6	1/10	21/210	4.7 ± 1.49	31.70	4.6 ± 1.30	28.26
	Mean	......	......	3.8 ± 1.36	35.79	3.7 ± 1.09	29.46
Marglobe	R5	1/10	21/210	9.5 ± 2.17	22.84	8.1 ± 2.28	28.15
	R17	1/10	29/290	6.1 ± 1.28	20.98	4.2 ± 1.50	35.71
	Mean	......	......	7.8 ± 1.74	22.31	6.2 ± 1.59	25.65
	RS16	......	10/100	......	......	7.5 ± 1.71	22.80
	RS7	2/20	34/298	6.7 ± 1.95	29.10	5.1 ± 1.89	37.06
	RS12	1/10	29/290	3.6 ± .69	19.17	4.9 ± 2.12	43.27
	Mean	......	......	5.2 ± 1.65	31.73	5.8 ± 1.22	21.03
	IR26	1/10	24/240	7.5 ± 1.96	26.13	6.2 ± 1.56	25.16
	IR13	1/5	24/120	7.2 ± 1.64	22.78	5.3 ± 1.64	30.94
	IA3	1/10	39/195	5.5 ± 1.08	19.64	4.4 ± 2.88	65.45
	Mean	......	......	6.7 ± 1.53	22.84	5.6 ± 1.30	23.21
	A15	2/20	22/220	0.7 ± 1.23	17.57	0.4 ± .65	162.50
	A1	1/8	10/80	2.0 ± .92	46.00	2.7 ± 1.32	48.89
	A6	1/10	21/210	2.8 ± .62	22.14	3.3 ± 1.02	30.91
	Mean	......	......	1.8 ± .79	43.89	2.1 ± .73	34.76

[a] See footnote 2 in the text. Letters indicating classes are described under the section in the text ''Methods and materials,'' and also apply to letters used in the parent strain number designations.

[b] Includes arithmetical mean of evaluations on individual plants and standard error.

[c] C.V. = Coefficient of variation.

[d] Numerator indicates number of cultures used, denominator indicates number of plants inoculated.

macrospores. If, however, the same number of isolates had been obtained from spores separated according to morphology, it is believed these minor differences would disappear. In RS7 the same degree of sectoring was noted in cultures of RS, IR, and IA character whether from microspores, macrospores, or chlamydospores. Thus, in these studies, morphology of a spore was apparently unrelated to the class of culture that developed from it.

PATHOGENIC VARIABILITY OF MONOSPORIC ISOLATES

Relative pathogenicity of the parent strains, as well as relative stability as indicated by absence or presence of sectors in cultures, has been reported (11). Briefly, the relative cultural stabilities of the strains included in these studies (Tables 4 and 5) are: R5 and A15 were most stable; some doubtful indications of variation were noted in cultures of A1 and A6; fairly definite sectors were noted in IA3 and IA13; sectors were more distinct in R17 and IR26; and the most divergence as exhibited by sectoring was seen in RS16, RS7, and RS12.

In experiments on pathogenic variability among monosporic isolates, parent strains and single spore isolates from these strains were tested for pathogenicity according to standard procedure (9). Affected plants were evaluated from 0, no apparent disease, to 15, early wilt and collapse caused by *Fusarium.* No intervening numerical points were recorded between 10 and 15. On the day all leaves on a plant become wilted, its stem is still upright and it is recorded as 10. During the next 2 or 3 days necrosis of the stem may progress to a complete and final collapse of this stem. Such a condition is given a rating of 15.

In the case of R5 (Table 4), considering the small number of plants, 10, used per treatment, it is believed that the standard error is not excessively large. High errors characterize the RS (most unstable) group, while the A (quite stable) group had relatively small errors.

In tests (Table 4) with the more susceptible and more delicate indicator plant Bonny Best, coefficients of variability were greater, on the whole, in the data obtained on the single-spore isolates from RS parents. Among the strains obtained from the intermediate group, IA3 gave the greatest coefficient of variability, which was interesting, since monosporic isolates from IA3 were of only 2 classes, IA and A (Table 2); but pathogenicities between these two types were considerably different in intensity. Of the 2 R strains the largest coefficient of variability was exhibited by R17, which produced saltations in culture, while R5 was free from sectoring.

The same general relationships found in the data from Bonny Best tests existed in the Marglobe tests. Standard errors, and coefficients of variation are not so high in the latter, a fact probably attributable to the greater resistance and more vigorous growth of those plants.

Strains of the pathogen that varied markedly in culture were similarly found to vary markedly in pathogenicity reactions. Likewise, degree of cultural variation was generally indicative of degree of range in pathogenicity.

TABLE 5.—*Pathogenic variation of tomato-wilt Fusarium between groups of monosporic isolates obtained from the same parent but differing in cultural classes*

Test host	Fusarium strain class and number[a]		Isolates used	Number of plants under each class				Chi-square[c]
				Pathogenicity classes[b]				
	Parent	Progenies	Plants inoculated	1	2	3	4	
Bonny Best	R5[d]	R odd	10/100[e]	0	0	11	89	.12
		R even	10/100	0	0	9	91	.12
	R17	R	6/60	0	12	35	13	19.40
		IR	6/60	0	29	26	5	.40
		IA	6/60	1	46	12	1	14.02
	RS7	RS	9/90	0	0	39	51	44.81
		IR	9/90	0	16	52	22	1.00
		IA	9/90	0	36	51	3	40.08
	RS12	RS	6/60	0	1	25	34	28.03
		IR	6/60	0	11	35	14	2.32
		IA	6/60	1	28	27	4	26.67
	IR13	IR	5/50	0	4	31	15	7.61
		IA	5/50	0	4	39	7	5.13
		A	5/50	0	22	28	0	19.93
	IA3	IA	15/150	0	1	49	100	53.19
		A	15/150	9	48	70	23	69.67
	A15[d]	A odd	10/100	86	14	0	0	4.00
		A even	10/100	94	6	0	0	1.88
Marglobe	R5[d]	R odd	10/100	0	9	65	26	3.62
		R even	10/100	0	8	48	42	2.71
	R17	R	6/60	0	43	17	0	2.86
		IR	6/60	0	40	20	0	5.37
		IA	6/60	6	50	4	0	15.56
	RS7	RS	5/42	0	27	15	0	4.91
		IR	5/42	0	32	5	5	10.78
		IA	5/42	3	28	11	0	6.70
	RS12	RS	5/50	0	29	21	0	7.16
		IR	5/50	0	38	11	1	.88
		IA	5/50	1	43	6	0	5.03
	IR13	IR	5/25	0	14	11	0	1.21
		IA	5/25	0	18	7	0	.53
		A	5/25	2	16	7	0	2.93
	IA3	IA	5/75	0	45	22	8	22.16
		A	5/75	32	33	28	12	36.92
	A15[d]	A odd	10/100	100	0	0	0	.00
		A even	10/100	100	0	0	0	.00

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[a] See footnote 2 in the text. Letters indicating classes are described under the section in the text "Methods and materials," and also apply to letters used in the parent strain number designations.

[b] 1 = none to very mild infection, 2 = mild, 3 = very severe disease and 4 = death. See also discussion of these classes in text under the section "Pathogenic variability of monosporic isolates."

[c] Tests made by standard methods using mean distributions of all progeny groups from each parent as theoretical values and testing distribution of separate groups for significant deviation therefrom. 5 per cent point = 7.82.

[d] Stable parent, progenies all like parent but divided arbitrarily into two groups: "odd" data from isolates listed under odd numbers, "even" data from those with even numbers.

[e] Numerator indicates number of cultures used, denominator indicates number of plants inoculated.

Chi-square tests also were made to study the differences in pathogenicity reactions in groups of single-spore isolates of different cultural character obtained from the same parental source. The single-spore isolates and their parents (Table 5) had been classified according to culture classes before they were used for inocula in these experiments. In arranging the data for statistical tests, results were used from the same number of cultures of a given class and compared with other class or classes that had been run for pathogenicity reaction at the same time. Selection of strains for these comparative tests was always strictly at random. The numbers of plants inoculated and all other conditions were as nearly identical as possible in these experiments. In applying the Chi-square test it was found that the 12 original evaluation classes, as described, did not give dependable frequency distributions. These were then grouped according to a method suggested in connection with the technique of testing for pathogenicity (9) the practicability of which was later demonstrated in handling data subsequently reported (11). Four classes of disease effects were therefore used, class 1, apparently no infection and very mild effect (disease evaluations 0, 1, 2); class 2, mild (3, 4, 5); class 3, serious (6, 7, 8); class 4, very severe disease and death (9, 10, 15). Differences among culture classes from the same parent source were tested by using the mean distribution of all groups within the parental source as the theoretical or calculated value.

Since it was impossible on the basis of cultural appearance to divide progenies of the stable R5 or A15 into different cultural groups within themselves (Tables 1 and 2), odd- and even-number isolates were arbitrarily combined for testing one against the other (Table 5). It will be seen that as might be expected both on mathematical grounds and judging from cultural similarity, odds and evens of R5 and A15, respectively, showed close agreement. It should be noted again, as was pointed out previously (11) and from the discussion in this paper, that parent cultures of the remaining R, RS, IR, and IA strains had all shown tendencies toward instability by developing frequent irregularities in cultural appearance. Also, as has likewise been noted above (Tables 1 and 2), monosporic isolates from these unstable cultures were of variable cultural types.

Monosporic isolates from R17 were grouped in R, IR, and IA classes. The R and IA groups were highly significant in their variation from the mean of the three (Table 5), but all 3 groups very evidently differed in their reactions. In RS7 and RS12, isolates were grouped in RS, IR, and IA classes. From both these strains the groups mentioned again differed in pathogenic reaction to a significant degree. Differences between groups also were found in those that came from 2 intermediate strains, IR13 and IA3.

In comparing the Bonny Best and Marglobe data, it is of interest that in reactions on Marglobe, the more resistant plant, the effects of different groups, on the whole, did not give such high Chi-square figures as did those from Bonny Best. For example, in RS12 tested on Bonny Best, the groups of single-spore isolates showed about the same comparability as in the RS7 monosporic-isolate groups. The Marglobe data, however, showed smaller Chi-squares among groups from RS12 than were observed in the RS7 material. In IR13 the monosporic isolates were in the IR, IA, and A groups. The A group had the highest Chi-square in the Bonny Best data. The IR and IA groups from this strain were not significantly different in pathogenic effects, and in the Marglobe data all 3 groups did not vary beyond limits that would be interpreted as significant. From IA3, groups IA and A, were tested. They differed so greatly that highly significant Chi-squares were obtained from both in the Bonny Best and Marglobe results. While Chi-squares from both tests were highly significant, the effect of greater resistance in Marglobe is again evident, for the Chi-squares are about half the size of those obtained from Bonny Best data.

The significance of reactions of differing groups within material from given strains demonstrates the variable nature of pathogenic reaction as it is correlated with cultural variability. Also, the most notably variant parent cultures yielded single-spore isolates showing the greatest variation in pathogenicity.

DISCUSSION

It appears from these studies that, in culture, the direction of change in the tomato-wilt Fusarium tends from the highly pathogenic types with light-color, abundant aerial growth, through classes with less aerial mycelium to the almost avirulent types having dark, appressed cultural characters with little or no aerial growth.

Moreover, the data indicate what may be expected through changes in *Fusarium* strains used as inocula in experiments on tomato resistance to Fusarium wilt, and emphasize the necessity of care in maintaining the more virulent forms of the parasite in question. Variants arise easily in certain cultures, can be perpetuated, and if accidentally used, may reduced disease severity and possibly vitiate gentic data based on the assumption that the inoculum was standardized in all of its effects throughout a long program of work.

These studies also further corroborate previously published findings (11), which indicated that the growing of tomato varieties of intermediate resistance may possibly be successful in some sections because of the presence of Fusarium types of low grade pathogenicity, while the same varieties grown in other sections, perhaps close by, may be unsuccessful because the strain of the parasite present may be highly pathogenic.

Results as reported herein and elsewhere (10, 11) also seem to explain one of the discrepancies in cultural characters that puzzled Haymaker (3) in his studies with the tomato Fusarium, and similar apparent disagreements that have occurred in other work with different fusaria. Haymaker obtained from White (12) isolates of the tomato-wilt Fusarium that differed markedly in cultural description from the original. After describing the differences Haymaker said: "Such a marked variation in results is difficult of explanation. . . . The change in the nature of the fungus may have been due to deterioration through prolonged growth in culture, but the fact that strain B had been cultured a year less than strain A would indicate that the probable cause of the change was the accidental transfer of saltation material not evident in test-tube cultures.'' His work emphasized questions that likewise are reflected in his statement regarding variability of the parasite and its effect on resistant strains of tomato: ''There is a remote possibility that variations in environmental conditions may be of importance, not alone in their effect upon the association of host and parasite, but also in inducing the fungus to change its inherent nature through general saltations.''

Haymaker described his strain ''A,'' grown on acidified potato-dextrose agar, as having fairly luxuriant, dark aerial mycelium, of salmon-pink to vinaceous color. This strain he obtained as White's strain ''60.'' White had described this strain, as it appeared on potato-dextrose agar, as faintly colored and producing some aerial growth. Both workers spoke of it as remarkably constant in cultural character. This is fairly typical of what we have observed in our studies (11) for an intermediate, raised, IR class of isolate. Although the IR types studied by us have been somewhat unstable in character, it is expected that further manipulation might result in segregating a more nearly stable strain. This was approached in the case of IR13–3 (Table 2).

Haymaker's strain ''B'' was white to pink on acid agar, with completely submerged mycelium in some cultures and powdery aerial growth in others; he considered it ''extremely variable.'' This ''B'' strain was from White's ''127,'' which White (12) described as having moderate aerial mycelium, faintly tinged with pink, and with colorless sclerotia. The specific mention of sclerotia by White, with his other characterizations, and Haymaker's observation on its notable variability, all agree with what we have found in our RS, unstable raised sclerotial types, and, as Haymaker surmised, is what might be expected from chance transfers from sectoring material. It appears that the behavior that Haymaker was troubled to explain (in the ''B'' strain's difference from its original character as White's ''127'') is the same phenomenon that was followed experimentally in the case of several series reported in the present paper (examples: RS16–4 and RS9–1, Table 2 and Fig.2). We also have found that strains relatively stable with respect to freedom from saltations, appear to retain this character throughout long cultivation under artificial conditions (examples: all R5, IR13–3, all A1, A6, and A15 cultures, and R21–3, Tables 1 and 2, and fig. 2).

It is of interest that monosporic cultures of the types RS, IR, IA, and A, but not R, were obtained from saltating RS strains, while all these culture classes including the R type were obtained from saltating R strains. Even though comparatively few RS cultures were secured from the R parents it seems significant that they could be obtained at all. A great many more monosporic isolates were obtained from RS parents; yet, the range of their variation did not include R classes. Obtaining all the various cultural classes from originally R-type cultures is important as an indication that these R strains may be considered basic in relation to the species concept, and may even have some bearing on general *Fusarium* classification.

The IR, IA, and A parents did not produce R or RS cultures through any of the handling methods resorted to in these studies. The relationship between RS and R types needs further and more exhaustive investigation.

Again it should be stated that, while change in cultural class character is, on the whole, towards a "downward" or appressed condition, in a very few cases (only 3 out of 2,031) variation seemed to occur in an upward direction. Such variation must take place in nature, since a wholly downward trend would eventuate in complete deterioration of a highly pathogenic population, which evidently does not happen in the field. Huge numbers of spores are produced by fusaria under ordinary conditions, and cultural studies with much larger numbers of isolates would possibly be more definitive with regard to upward variation. The comparatively few numbers of such variants indicated in our studies might be entirely sufficient to multiply increasingly virulent strains to cause severe epidemics where, before, disease may have been consistently present but only moderate in effect.

It is obvious from these studies also that, with the range and general direction of change understood, a more intelligent view is possible of the group of culture types that are represented by *Fusarium bulbigenum* var. *lycopersici*. In a previous report (11) a strain, No. 30, was noted that produced coloration that diffused from mycelia into the surrounding agar. Many other types of variation probably will be found; indeed, these that have been described may be only a small proportion of what actually occurs.

SUMMARY

Studies were made of over 2,000 single-spore isolates from many parent strains of the tomato-wilt Fusarium (*F. bulbigenum* var. *lycopersici* (Brushi) Wr. and R.). In the material used, monosporic isolates vary most in cultural type and pathogenicity when obtained from parents known to be most commonly of saltating nature, and vary least from parents that are apparently free from sectoring in culture.

Variation, with only a few exceptions, tends in the direction from cultures with abundant aerial mycelium to those with growth that is almost, if not completely, appressed to the surface and submerged in the agar or liquid. The raised form is the most highly pathogenic in character, the appressed is mildest, and intermediate cultural types are intermediate in pathogenicity.

Out of 2,031 monosporic isolates 3 were secured in which variability was towards a more raised type of growth than characterized their parents.

Variation in culture classes was independent of the choice of microspores, macrospores or chlamydospores for isolation studies.

It is possible to secure the whole gamut of cultural variants from certain strains of fully raised character that have at the same time a variable habit resulting in saltations. No other type of strain, whether so-called raised

sclerotial, intermediate raised or appressed, or completely appressed, yielded all 5 classes of cultures. Strains classed as raised, but variant, are considered the basic type whence all other strains are obtainable.

By isolating single spores from sectors, and by selection through successive generations of single-spore reisolations, strains of the *Fusarium* with little or no aerial mycelium (low pathogenic type), were obtained from parent cultures that had abundant mycelium in culture (highly pathogenic type).

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LITERATURE CITED

1. EDGERTON, C. W., and C. C. MORELAND. Tomato wilt. La. Agr. Exp. Stat. Bull. 174. 1920.
2. EIDE, C. J. The pathogenicity and genetics of *Gibberella saubinetii* (Mont.) Sacc. Minn. Agr. Exp. Stat. Tech. Bull. 106. 1935.
3. HAYMAKER, H. H. Pathogenicity of two strains of the tomato-wilt fungus, *Fusarium lycopersici* Sacc. Jour. Agr. Res. [U.S.] 36: 675–695. 1928.
4. LEONIAN, L. H. Studies on the variability and dissociations in the genus *Fusarium*. Phytopath. 19: 753–874. 1929.
5. ————. The pathogenicity and the variability of *Fusarium moniliforme* from corn. West Virginia Agr. Exp. Stat. Bull. 248. 1932.
6. PORTE, W. S., and F. L. WELLMAN. Development of interspecific tomato hybrids of horticultural value and highly resistant to Fusarium wilt. U. S. Dept. Agr. Circ. 584. 1941.
7. SNYDER, W. C. Variability in the pea-wilt organism, *Fusarium orthoceras* var. *pisi*. Jour. Agr. Res. [U.S.] 47: 65–88. 1933.
8. ULLSTRUP, A. J. Studies on the variability of pathogenicity and cultural characters of *Gibberella saubinetii*. Jour. Agr. Res. [U.S.] 51: 145–162. 1935.
9. WELLMAN, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopath. 29: 945–956. 1939.
10. ————, and D. J. BLAISDELL. Differences in cultural characters and pathogenicity of strains of tomato-wilt Fusarium. (Abstract) Phytopath. 29: 24. 1939.
11. ————, ————. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bull. 705. 1940.
12. WHITE, R. P. Studies on tomato wilt caused by *Fusarium lycopersici* Sacc. Jour. Agr. Res. [U.S.] 34: 197–239. 1927.

FURTHER EVIDENCE FOR MULTIPLICATION OF THE ASTER-YELLOWS VIRUS IN THE ASTER LEAF HOPPER

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INTRODUCTION

This article contains an expanded account of work briefly reported earlier (3) and a complete treatment of experiments that have since been carried out. The tests described show that the aster-yellows virus, recently named *Chlorogenus callistephi* H. var. *vulgaris* H. (7), can be mechanically transferred from insect to insect, that the virus undergoes an incubation period in the inoculated insects, and that once the latter become infective they are able to transmit the virus for many days. Evidence is presented indicating that the aster-yellows virus multiplies in its insect vector. Certain other properties of the virus have also been elucidated.

In 1902 R. E. Smith (14) gave a good description of the symptoms of aster yellows. In 1924 Kunkel (8) discovered that the leaf hopper, *Macrosteles divisus* Uhler, transmitted the causal agent of the disease. Later (9) he adduced evidence of the virus nature of the pathogen and made comprehensive studies on its relationship to the insect vector and numerous plant hosts. The virus was transmitted from plant to plant by grafting, but all attempts at mechanical transmission failed. Severin (13) in 1934 stated that "California aster and celery yellows has been transmitted on rare occasions from a feeding solution containing crushed infective *Cicadula divisa* bred on diseased plantain or ribgrass (*Plantago major*)." So far as the writer is aware, Severin has published no further information on these feeding experiments with the aster-yellows virus. With the introduction, by Rawlins and Tompkins (12), of carborundum as an abrasive in plant-virus inoculations, the mechanical transmission of many plant viruses was greatly facilitated. Rawlins and Tompkins, however, failed to transmit aster-yellows virus by this means.

The writer's attempts to transmit aster-yellows virus mechanically from insect to insect at 0° C. were based on a number of facts and hypotheses. Storey (16) demonstrated that an insect could be successfully inoculated with the virus for which it is a vector. By means of pin punctures and injections through fine glass capillaries, he succeeded in transferring the maize-streak virus, *Marmor maidis* H. var. *typicum* H., from the leaf hopper, *Cicadulina mbila* Naude, to other leaf hoppers of the same species. He also transmitted the virus from plant to leaf hopper. The maize-streak virus has not been transferred mechanically from plant to plant. The work of Storey suggested that similar techniques of insect inoculation might be successfully applied to the aster-yellows virus. The work of Kunkel (10) on heat-induced incubation periods in viruliferous aster leaf hoppers affords good evidence that the aster-yellows virus is very unstable, because a treatment for 12 days at 32° C. appeared to destroy the virus within the insect. As most viruses are unstable in juices of their hosts at ordinary temperatures, while they are apparently stable *in vivo* at the same temperatures, Kunkel's data suggest that the aster-yellows virus might be rapidly inactivated at ordinary temperatures during mechanical transmission. For this reason the transmission of aster-yellows virus from insect to insect was attempted at 0° C. rather than at room temperature. Kunkel interpreted the heat-induced incubation periods as being due to inactivation of part of the virus during the heat treatment and multiplication at lower temperatures. Because this interpretation appeared most probable to the writer, it seemed logical to expect that, if a small amount of the virus were successfully introduced into the leaf hopper, it might increase until the insect became infective.

MATERIALS AND METHODS

Nonviruliferous insects were hatched and reared on rye, which is immune from the aster-yellows virus (9). For convenience, the insects used as a source of virus will be designated as "source" insects. For some purposes the source insects were hatched and reared on diseased asters, for others they were fed for only a limited time on diseased plants. Usually, 50 live source insects in a 125-cc. Erlenmeyer flask were weighed on a balance sensitive to changes of 0.1 mg. These insects were then placed in a room held at – 10° C. just long enough to render them inactive but not long enough to freeze them. They were then taken to a room held at 0° C. where all subsequent procedures connected with inoculation were carried out. This room contained a sample of nonviruliferous young adult leaf hoppers of about the same age and all the virus-free apparatus and materials necessary for the inoculation. The insects inoculated will hereafter be referred to as "test" insects. If so desired, the inactivated source insects were counted and then thoroughly ground by means of a glass mortar and pestle. Before the macerated pulp had time to dry, a calculated volume of 0.85 per cent saline solution was placed in the mortar to give the dilution of juice desired. For the purpose of calculating dilutions, the whole weight of the leaf hopper was assumed to be a liquid having the same density as water. The actual dilution of the virus-containing fluids from the insect was, therefore, greater than stated. Preparations designated as "undiluted" really had 2 or 3 drops of distilled water added to the pulp so that the material could be manipulated. Inoculations with the undiluted solutions, which were not centrifuged, were made with an insect pin dipped in the mass of ground insects. Three minutes' centrifugation of the diluted solutions in an International Clinical Centrifuge was sufficient to throw most of the coarse particles to the bottom and leave a supernatant clear enough not to clog the fine capillaries used for injection. About 10 of the test insects were placed in an enamel pan 20 cm. in diameter and 7 cm. in depth. The infectious solution was drawn up into a glass capillary and a small amount forced into the test insect through a puncture in the abdomen. The insect was pushed off the capillary tube by means of an insect pin mounted on a slender wooden rod. Ordinarily, 120 insects were inoculated with each solution. In some of the early experiments a single insect was injected as often as 5 times. It was soon discovered, however, that a single injection was the most satisfactory procedure. After the test insects were inoculated, they were placed in a 125-cc. Erlenmeyer flask and allowed to come to room temperature over a period of about 10 minutes.

The group of inoculated test insects was then placed on a single large aster plant (*Callistephus chinensis* Nees) or on young rye plants (*Secale cereale* L.). If placed on an aster plant, they were removed from this plant before they became infective and were tested individually on a series of small aster plants. If the test insects were placed on rye plants, they were transferred as a group once a week for 3 weeks to a cage containing fresh rye plants and then tested either individually or in small colonies on a succession of small aster plants. To avoid the accidental transfer of nymphs produced by the test insects, each test insect or colony was held on each aster plant for 1 week, except in the case of the last test plant. The insect or colony was held on the last plant for 2 weeks. All aster plants were observed in a greenhouse for 28 days after the removal of the insects. During transfers, the noninoculated control insects were transferred first, then the test insects from the highest dilution group to the lowest, and lastly the inoculated control insects. Since rye is immune from the virus, it was advantageous to hold the insects on rye while the virus passed through the long and variable incubation period in the insect. Satisfactory results were obtained with the rye only when it was grown in sand and fed a complete mineral nutrient solution. The rye seed employed was always carefully examined and weed seeds removed. The transfer to fresh rye plants every week made certain that unsuccessfully inoculated insects could not pick up the virus from a rare weed that might have escaped detection and might have been successfully inoculated by insects that had already become infective.

With the exception of some of the first experiments, an attempt was made to keep both source insects and test insects at a temperature of 24° C. or

TABLE 1.—*Transmission of aster-yellows virus by individual leaf hoppers inoculated with various dilutions of juice from viruliferous insects*

Insects inoculated with	Insect number	Days insects held on successive aster plants																	
		1–10	11–17	18–24	25–31	32–38	39–44	45–50	51–61	62–66	67–73	74–80	81–86	87–93	94–100	101–107	108–114	115–120	121–129
Undiluted juice	1	–	–	–	+	+	+	–[a]											
	2	–	–	+	+	+[a]													
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–[a]
	4	–	–	–	–	–	–	–	–	–[a]									
	5	–	–	–	–	–	–	–	–	–	–	–	–	–[a]					
Juice at 10^{-1}	1	–	–	–[a]															
	2	–	–	–	–	–	–	–	–[a]										
	3	–	–	–	+	+	+	+	+	–	–[a]								
	4	–	–[a]																
	5	–	–	–	–	+	+[a]												
	6	–	–	–[a]															
	7	–	–	–	–[a]														
Juice at 10^{-2}	1	–	–	–	+	+	+[a]												
	2	–	–	–	–	–	–	–[a]											
	3	–	–	–	–	–	–	–	–[a]										
	4	–	–	–	+	+	+	+	+[a]										
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–[a]		
Noninoculated controls		No infections with 15 insects																	

[a] Insect died.
+ = plant developed aster yellows.
– = plant remained healthy.
Incubation period in insect: 17–31 days.

lower in order to avoid any heat-induced inactivation of the virus within the insect.

MECHANICAL TRANSMISSION

After it had been shown that the aster-yellows virus could be transmitted mechanically from insect to insect, an experiment was undertaken to ascertain to what extent the juices of source insects could be diluted in 0.85 per cent salt solution and still remain infective. Undiluted juice, juice diluted 10^{-1}, and juice diluted 10^{-2} were inoculated into different groups of 25 test insects. The inoculated groups and a control group of 25 noninoculated insects were each confined to a single large aster plant for 10 days. Then the surviving insects were transferred individually to a succession of aster plants at the intervals shown in table 1 until they died. The results of this experiment indicated that the juice of the source insects was infectious up to a dilution of 10^{-2}. It was clear also that the virus underwent an incubation period in the infective insects, the minimum incubation period varying from 17 to 31 days. With one exception (insect 3, dilution 10^{-1}), it is probable that the infective insects transmitted the virus until they died.

The experiment was repeated, testing higher dilutions and inoculating 100 insects with each dilution instead of 25. Each group of insects was held on a single large aster plant for 11 days and then the survivors were tested individually on a succession of aster plants during the following 76 days, at which time all but 9 of the insects had died. In table 2, data from this experiment show that the source insect juice was infective at dilutions up to 10^{-2} but not at higher dilutions. In table 3, the data on the infective insects in the group inoculated with dilution 10^{-1} are presented in detail. The incubation period in these insects varied from 11 to 45 days. It seemed probable that every infective insect transmitted the virus until it was dead or dying.

TABLE 2.—*Transmission of aster-yellows virus by individual leaf hoppers inoculated with various dillutions of juice from viruliferous insects*

Dilution of viruliferous insect juice	Number of insects tested individually on aster plants	Number of insects infective	Number of test plants employed	Number of plants infected
10^{-1}	52	11	184	44
10^{-2}	28	3	58	8
10^{-3}	32	0	96	0
10^{-4}	30	0	110	0
10^{-5}	38	0	176	0
Noninoculated insects	68	0	309	0

These experiments showed that the juice of source insects was infectious up to dilutions of 10^{-2} in saline solution and that the virus underwent a minimum incubation period in the inoculated insects of from 11 to 45 days. The experiments also indicated that once the inoculated insects became infec-

TABLE 3.—*Transmission of aster-yellows virus by individual leaf hoppers inoculated with a 10^{-1} dilution of juice from viruliferous insects*

Insect number	Days insects held on successive aster plants									
	1–11	12–24	25–31	32–38	39–45	46–52	53–59	60–66	67–73	74–81
1	–	–	–	–	+	+	+	+	–[a]	
2	–	–	–	+	+	+	+[a]			
5	–	–	–	–	–	+	[b]	+	–[a]	
11	–	–	–	+	+	+	–[a]			
12	–	–	+	+	+	+	+	+	+	+[a]
19	–	+	+	+	+	+	+	+	+	+[a]
28	–	–	+	+[a]						
30	–	–	+[a]							
40	–	–	+	+[a]						
44	–	–	+	+	+	+	+	+	+	+[a]
45	–	–	+[a]							

[a] Insect died.
[b] Plant died due to fungous infection.
+ = plant developed aster yellows.
– = plant remained healthy.
Incubation period in insect: 11–45 days.

tive they were able to transmit the virus to asters until they died, sometimes as long as 50 days later.

In the first experiments, the percentage of infective insects varied from 6 to 11 per cent of the insects inoculated and from 14 to 46 per cent of the insects surviving at any one time. Experiments were conducted in an attempt to improve the efficiency of inoculation. In the first experiment, the juice of source insects diluted to $10^{-1.5}$ was injected into the thorax of 50 insects just in front of the last pair of legs and into the abdomen of a second group of 50 insects just behind the last pair of legs. These inoculations were carried out at 0° C., as heretofore. A third group of 50 insects was inoculated in the same manner as the first group, except that the whole procedure was carried out at 25° C., using ether to anaesthetize the test insects. The inoculated insects were held on rye plants for 3 weeks and then tested individually on asters for 4 weeks. The results of the experiment (Table 4) indicated that the inoculations at 0° C. were much more successful than the inoculations at 25° C. Injections in the abdomen were easier to make than those in the thorax, and the results suggested that they were more efficient.

In a second experiment, groups of 120 test insects were treated in various ways. One group was held uninoculated as a control. A second control group consisted of insects inoculated with infectious juice diluted to $10^{-1.5}$. Half of the insects were injected with as large a volume of inoculum as could be forced into their bodies without killing too many of them. The remaining 60 insects were injected with as small a volume of inoculum as could be detected entering their bodies, *i.e.*, the meniscus in the capillary was forced to move only very slightly at each injection. A third group of insects was inoculated with juice diluted $10^{-1.5}$ that had been stored 24 hours at 0° C. The fourth group of insects was inoculated with the same dilution of juice, but the source insects from which the juice was taken had been

TABLE 4.—*Transmission of aster-yellows virus to leaf hoppers by different methods of mechanical inoculation*

Experiment	Treatment	Insects injected	Insects tested	Insects infective	Percentage of insects infective
A6a	25° C. Ether anaesthesia	50	28	7	25
	0° C. Injected in thorax	50	37	28	76
	0° C. Injected in abdomen	50	40	35	88
A6c	Noninoculated controls	120	79	0	0
	Inoculated controls:				
	60 with large volume of inoculum	60	16	15	94
	60 with small volume of inoculum	60	29	22	76
	Total inoculated controls	120	45	37	82
	Inoculated with juice held 24 hrs. at 0° C.	120	56	2	4
	Inoculated with juice from frozen insects	120	78	2	3

frozen for 20 minutes at −10° C. The test insects were held on rye plants for 3 weeks and were then tested on aster plants for 4 weeks. The results of this experiment (Table 4) indicated that most but not all of the virus was destroyed by freezing the source insects or by storing the juice at 0° C. for 24 hours. Injection of large volumes of inoculum resulted in a higher percentage of infective insects among the survivors than when a small injection was employed. However, the higher mortality in the former group resulted in a smaller actual number of infective insects.

MULTIPLICATION OF VIRUS IN THE INSECT

The writer has been primarily interested in the technique described above because it should make possible several types of experiment bearing on the question of whether or not the aster-yellows virus increases in its insect vector.

A conclusive demonstration that a plant virus is able to multiply in its insect vector has several important implications. It would be strong evidence for the relationship between plant and animal viruses because a virus capable of multiplying in both plants and animals would be, in a very real sense, both a plant virus and an animal virus and would serve as a connecting link between the two groups. Such viruses would raise interesting questions regarding their position in any system of virus classification. Moreover, multiplication in the insect vector would probably be involved in the specific relationships between certain plant viruses and their insect vectors (11), the possible vectors of certain viruses being restricted in part at least to those insects in which the virus was able to multiply. That this may not be the only factor involved seems probable from the demonstration by

Bennett and Wallace (2) that the sugar-beet curly-top virus (*Chlorogenus cutetticola* H.) may be retained in non-vector insects for as long as 3 weeks. Storey's (16) demonstration that inactive races of *Cicadulina mbila* can be rendered infective by puncturing insects that have ingested maize-streak virus indicated that purely physical barriers in the intestinal wall were also involved in the specificity of vectors. Lastly, if a virus is capable of multiplying in both plants and animals, any theory of virus multiplication would have to account for such increase. For example, the precursor hypothesis (15) seems to imply that in any medium supporting virus multiplication there exists a large element similar to the virus, which, on undergoing a change involving a comparatively small fraction of its total structure, becomes the active virus. If this be true, it would seem necessary to suppose that the same precursor elements occur in as widely separated organisms as insects and plants. While this may be so, the possibility that viruses grow by some process akin to the assimilation of simple compounds by organisms deserves consideration.

The discovery that the aster-yellows virus can be readily transferred mechanically from insect to insect makes possible several types of experiments on the relationship between virus and vector. For example, the technique makes possible experiments measuring any increase of virus that might occur in insects. Similarly, it permits experiments designed to determine whether or not there is virus inactivation in insects held at high temperatures and it makes feasible attempts to prove virus increase in insects by means of serial passages. The experiments on multiplication described in this paper are of the first type.

In the first of these experiments, a large nonviruliferous colony of young adult leaf hoppers of about the same age was placed on diseased aster plants that had been stripped of all leaves but those showing pronounced symptoms of aster yellows. Twenty-four hours later the asters were removed and the insects subsequently maintained on rye plants that were changed at intervals of 9 days or less. It should be emphasized here that the only virus these insects ever obtained from plants was that secured during the 24 hours they were on diseased asters. All intervals in this and subsequent experiments were reckoned from the day on which the insects were placed on diseased aster plants, this day being called "1," the next "2," and so on. On certain days thereafter some of these insects were withdrawn at random from the colony and ground. The juice, diluted with saline, was centrifuged and the supernatant solution injected into test insects. Since the source insects gradually increase in weight during the course of an experiment such as this, dilutions were based on the average weight of a single leaf hopper as determined on the day when the first titration was made. The average weight of 302 leaf hoppers on the third day after they were placed on diseased asters was 0.706 mg. In subsequent titrations the number of insects in the sample was multiplied by 0.706 and the weight thus calculated used to determine the volume of saline required to give the desired dilution. A fresh sample of 100 source insects was employed for each test with undiluted juice and with juice diluted 10^{-1}. Fifty source insects were used for each test with juice diluted 10^{-2}. One hundred and twenty test insects were inoculated with each dilution. The inoculated test insects were held on rye for 3 weeks and then tested individually on asters for 4 weeks.

TABLE 5.—*Transmission of aster-yellows virus by individual leaf hoppers inoculated with various dilutions of juice from insects fed on diseased aster plants for 1 day*

Day during which juice from source insects was injected into test insects[a]	Ratio: $\frac{\text{Number of insects infective}}{\text{Number of insects tested}}$			
	Dilution of juice from source insects injected into test insects			Control insects noninoculated
	10^0	10^{-1}	10^{-2}	
3	0/50	0/50	0/50	0/50
4	0/50	0/50	0/50	0/50
10	0/50	0/46	0/48	0/49
17	0/29	0/9	0/23	0/50
24	0/41	0/32	0/29	0/50
31	0/1	1/8	2/47	0/50

[a] The day during which the source insects fed on diseased aster plants was designated "1." The following day was designated "2," and so on.

The results of the first experiment (Table 5) were far from conclusive. Positive results were not obtained until the 31st day, when 1 infective insect appeared at dilution 10^{-1} and 2 at dilution 10^{-2}. All noninoculated, control insects remained noninfective. This experiment was carried out in midsummer, and later experience indicated that some of the negative results were due to heat inactivation of the virus in the insects.

The experiment was repeated in the autumn with some changes. A large nonviruliferous colony of young adults was fed on diseased aster plants for 48 hours and was then placed on rye plants. Whenever titrations were run on these source insects, a group of controls inoculated with juice from insects that had fed all their lives on diseased plants, was included. The controls served as a check on the technique of inoculation on the day in question and also on the possible inactivating effect of the temperature at which the test insects were held subsequent to their inoculation. The temperature at which the insects were kept was held at 24° C. or lower. In this second experiment, the source insects were titrated for their virus content on the 8th and 10th days. The results of the experiment, presented in table 6, show that on the 8th day the juice of the source insects was infectious at a dilution of 10^{-2}. Positive results were also obtained on the 10th day, but it was unsafe to conclude that the increased number of infective insects obtained on this day resulted from an increase of the virus in the source insects. The negative results with undiluted juice, even though a high percentage of inoculated insects survived, are attributed to the inefficiency of the pin-puncture method of inoculation. The experiment showed that virus had reached a high concentration in the insects at least 2 days before they would have been

TABLE 6.—*Transmission of aster-yellows virus by individual leaf hoppers inoculated with various dilutions of juice from insects fed on diseased aster plants for 2 days*

Day during which juice from source insects was injected into test insects[a]	Ratio: $\frac{\text{Number of insects infective}}{\text{Number of insects tested}}$					Ratio: $\frac{\text{Number of plants infected}}{\text{Number of test plants}}$				
	Dilution of juice from source insects injected into test insects			Control insects		Dilution of juice from source insects injected into test insects			Control insects	
	10^0	10^{-1}	10^{-2}	Noninoculated	Inoculated with infective insect juice undiluted	10^0	10^{-1}	10^{-2}	Noninoculated	Inoculated with infective insect juice undiluted
8	3/79	0/9	3/42	0/86	8/65	7/221	0/27	9/110	0/149	19/183
10	0/74	9/28	21/83	0/93	8/91	0/197	16/64	40/142	0/149	13/148

[a] The day during which the source insects fed on diseased aster plants was designated "1." The following day was designated "2," and so on.

able to infect plants by feeding. This is confirmatory of Storey's observation (16) that the maize-streak virus could be detected in the vector some 6 hours before the insect became infective.

The experiment with further modifications was carried out a third time. In this case titrations for virus in the source insects were made at dilutions of 10^{-1}, 10^{-2}, and 10^{-3}. Moreover, instead of testing the inoculated insects individually on asters, they were tested in colonies of 5, unless the survivors numbered less than 50, when they were divided in such a way as to give as many colonies as possible up to 10. The results of this experiment (Table 7) were not conclusive. Although all noninoculated controls remained noninfective and a high percentage of inoculated control colonies became infective, in the actual titrations only 3 infective colonies (probably only 3 infective insects) appeared. One of these occurred on the 8th day at dilution 10^{-3} and 2 on the 11th day, 1 at dilution 10^{-2} and 1 at dilution 10^{-3}. The poor results are attributed to the fact that the source insects did not pick up much virus. This was shown by testing 50 source insects individually on asters for 2 days, 42 days after they had fed on the diseased asters. Only 2 of the 50 aster plants became diseased; 50 noninoculated control plants remained healthy. The high mortality in insects inoculated with juice from the source insects also contributed to the poor results. Juice used to inject the inoculated controls was always diluted $10^{-1.5}$, yet it consistently resulted in a lower mortality in the injected insects than the juice from the source insects diluted to 10^{-2}. The reason for this effect is unknown.

TABLE 7.—*Transmission of aster-yellows virus by colonies of from 1 to 5 leaf hoppers inoculated with various dilutions of juice from insects fed on diseased aster plants for 1 day*

Day during which juice from source insects was injected into test insects[a]	Ratio: $\frac{\text{Number of colonies infective}}{\text{Number of colonies tested}}$				
	Dilution of juice from source insects injected into test insects			Control insects	
	10^{-1}	10^{-2}	10^{-3}	Noninoculated	Inoculated with infective insect juice diluted $10^{-1.5}$
2	0/18	0/10	0/15	0/18	17/17
3	0/7	0/17	0/11	0/17	15/15
5	0/6	0/10	0/17	0/18	14/15
8	0/8	0/8	1/15	0/17	9/10
11	0/5	1/9	1/10	0/19	16/16
15	0/10	0/10	0/10	0/19	15/15
22	0/10	0/10	0/14	0/17	19/20
41	0/10	0/10	0/10	0/15	16/16

[a] The day during which the source insects fed on diseased aster plants was designated "1." The following day was designated "2," and so on.

In the fourth repetition of the experiment, further improvements were introduced. The source insects were kept on large healthy aster plants both before and after being placed for 1 day on diseased plants. Before the

TABLE 8.—*Incubation period in source insects that were titrated for their virus content on various days*

Plants	Days before feeding on diseased aster plants[a]	Days after feeding on diseased aster plants																									
	−2 to −7	2–5	6–7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Number infected	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Number used	15	12	12	6	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

[a] The day during which the source insects fed on diseased aster plants was designated "1." The following day was designated "2," and so on. The days preceding that on which the insects were fed on diseased asters were given negative numbers beginning with "−2."

TABLE 9.—*Transmission of aster-yellows virus by colonies of 5 leaf hoppers inoculated with various dilutions of juice from insects fed on diseased aster plants for 1 day*

Day during which juice from source insects was injected into test insects[a]	Ratio: Number of colonies infective / Number of colonies tested					Calculated probable percentage of infective insects					Ratio: Number of plants infected / Number of test plants				
	Dilution of juice from source insects injected into test insects			Control insects		Dilution of juice from source insects injected into test insects			Control insects		Dilution of juice from source insects injected into test insects			Control insects	
	10^{-1}	10^{-2}	10^{-3}	Noninoculated	Inoculated with infective insect juice diluted 10^{-2}	10^{-1}	10^{-2}	10^{-3}	Noninoculated	Inoculated with infective insect juice diluted 10^{-2}	10^{-1}	10^{-2}	10^{-3}	Noninoculated	Inoculated with infective insect juice diluted 10^{-2}
2	0/19	0/20		0/17	17/19	0	0		0	36	0/38	0/39		0/34	25/38
4	0/19	1/19		0/18	18/18	0	1		0	> 44	0/38	2/38		0/36	33/34
8		3/20	1/20	0/19	19/19		3	1	0	> 45		4/40	1/40	0/37	33/38
12		13/14	18/18	0/18	20/20		41	> 44	0	> 45		23/28	29/36	0/36	36/40
16		7/24[b]	9/19	0/17	17/17		29	12	0	> 43		11/44	11/38	0/34	31/34

[a] The day during which the source insects fed on diseased aster plants was designated "1." The following day was designated "2," and so on.

[b] In this case only 24 insects survived and they were tested individually on asters.

incubation period had elapsed, these healthy aster plants were changed daily. In this way the minimum incubation period for the whole colony (rather than for a sample) was accurately ascertained. It was found that the insects began to infect the aster plants during the 18th day (Table 8). In addition, samples of 50 insects were withdrawn from the colonies on the 15th and 32nd days and tested individually on asters for 1 day. None of the insects tested on the 15th day was infective, but on the 32nd day 15 insects (or 30 per cent) infected aster plants. None of 100 noninoculated control aster plants in these two tests became diseased.

Titrations were made on the 2nd, 4th, 8th, 12th, and 16th days, using selected dilutions as shown in table 9. In the titrations, 50 source insects were used to prepare each dilution. For the purpose of making dilutions, the average weight of a single source insect at the beginning of the experiment was taken as 0.857 mg. and was based on the weights of 3 samples of 50 insects each. An effort was made to inject about the same amount of solution into each test insect. After holding the inoculated test insects, and both kinds of control insects, on rye for 3 weeks, they were tested in colonies of 5 on aster plants (with one exception mentioned in table 9). Each colony was held on 1 aster plant for 1 week and on a 2nd aster plant for the next 2 weeks. The noninoculated control insects did not transmit the virus, whereas almost 100 per cent of the inoculated control colonies became infective. The latter had been inoculated with a 10^{-2} dilution of juice from insects that had fed all their lives on diseased asters. Entirely negative results were obtained on the 2nd day at dilutions 10^{-1} and 10^{-2}. On the 12th day, a high proportion of the colonies was infective at dilutions 10^{-2} and 10^{-3}. Smaller numbers of infective colonies were obtained on the other days. From the data on colonies, it is possible to calculate the probable number of infective insects in any group. Such calculations have been made and the data are included in table 9.[1] It is apparent from the data presented in table 8 that the source insects could have received no virus from plants other than that secured during the 1 day on diseased aster plants. The data of table 9 indicate that the virus in the insects increased at least 100-fold between the 2nd and the 12th day. The data for the 16th day suggest that the virus concentration decreased between the 12th and 16th days. These results indicate that the virus multiplied in the insects and that it reached a very high concentration 6 days before the incubation period had elapsed.

DISCUSSION

The technique of transferring the aster-yellows virus from insect to insect promises to be a useful tool for investigating some properties of the aster-yellows virus that have hitherto eluded determination. Similar techniques may be successful with still other plant viruses transmitted by leaf hoppers. Transmission at 0° C. may be an important factor in the mechanical transfer of unstable viruses. It is believed to be an important consideration in the above experiments on virus multiplication in the vector.

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[1] The writer wishes to acknowledge his indebtedness to Dr. W. J. Youden for his assistance in making these calculations.

Evidence for the multiplication of a plant virus in its insect vector already exists. In 1935, Fukushi (5) obtained transmission of the rice dwarf-disease virus (*Marmor oryzae* H.) from insect to insect through the egg to the third generation and interpreted this result as evidence of virus multiplication in the vector. Storey (17) suggested that explanations other than multiplication may account for this phenomenon, and Bawden (1) advanced several arguments against Fukushi's interpretation of his data. Fukushi's recent work (6) showing that the virus may pass from insect to insect through the egg to the 7th generation considerably strengthens his interpretation.

Kunkel (10) found that, by holding viruliferous aster leaf hoppers at 32° C., the insects lost their ability to transmit aster-yellows virus either temporarily or permanently, depending on the length of time they were held at the high temperature. He was unable to observe any injurious effect of the heat treatment on insects held at even higher temperatures, and showed that insects, rendered noninfective by heat treatment, could reacquire and transmit the virus (11). He suggested that the heat treatment inactivates all or part of the virus and that insects that temporarily lose their ability to transmit aster-yellows virus regain the ability by multiplication of the remaining virus until an infective concentration is reached. Bawden (1) advanced several other interpretations and stated that "there are so many possible alternative explanations of these results that it would seem extremely premature to regard them as evidence for the multiplication of the virus in the vector." While each of Bawden's alternative explanations for the evidence of Fukushi and Kunkel is possible, it should be remembered that there is no experimental evidence to show that they actually apply in either case. Bawden apparently accepts the evidence of Freitag (4) and Bennett and Wallace (2) as showing that the beet curly-top virus does not multiply in the beet leaf hopper, *Eutettix tenellus* (Baker). It is possible, of course, that the viruses of aster yellows and rice dwarf disease multiply in their vectors, even although the virus of curly top of beets may not multiply in *E. tenellus.* The evidence against the multiplication of the curly-top virus in its vector consists of the fact that infective leaf hoppers frequently lose their ability to infect without fresh access to diseased plants, the demonstration that the length of time the insects remain infective is proportional to the length of time they feed on diseased plants, the evidence that the virus content of infective insects decreases with the passage of time after they feed on diseased plants, and the recognition of the ability of insects that have lost their infectivity to reacquire and transmit the virus. Storey (17) has pointed out the possibility that insect-borne viruses might infect their vectors locally instead of systemically. Increase of the virus might be restricted by the number of cells invaded during the feeding period and might well be limited in a number of ways to a certain amount per cell. The latter hypothesis cannot be considered untenable by the evidence so far accumulated indicating that the curly-top virus does not multiply in the beet leaf hopper.

In the present work, direct evidence has been presented in favor of the multiplication of the aster-yellows virus in the aster leaf hopper. It should be emphasized that in the titration experiments the whole insects were ground up and not just a part of them. It seems unlikely, therefore, that the results could be due to a shift in the location of the virus within the insects. The indications of a decrease in virus concentration after a peak is reached are believed to be real. If it is an actual decrease, it seems that a part of the incubation period is required for multiplication and that another part of the incubation period is necessary for transfer of the virus from locations where it multiplies to other locations from which it can enter the plant. In virus diseases of animals the virus is commonly detectable in increasing amounts during a certain period following inoculation, and then, after reaching a peak, the amount that can be detected commonly decreases, frequently to a point where no virus at all can be detected. Equine encephalomyelitis affords a good example of this phenomenon (18). It seems probable to the writer that the aster-yellows virus first increases and then decreases in all aster leaf hoppers. In some the decrease may proceed far enough so that the insect loses its ability to transmit unless it has access to a fresh source of virus. It should be pointed out that the reacquisition of a virus disease by animals is not at all unusual (19).

SUMMARY

The aster-yellows virus has been mechanically transmitted from insect to insect. Juice from viruliferous insects is infectious at dilutions as high as 1:1000 in 0.85 per cent NaCl solution.

The virus in insects inoculated by mechanical means underwent a minimum incubation period varying from 11 to 45 days.

Once the inoculated insects began to transmit the virus, they usually did so until they died.

Mechanical transmission of the virus at 0° C. was more efficient than mechanical transmission at 25° C.

Freezing viruliferous insects for 20 minutes at $-10°$ C. destroyed most of the virus.

Most of the virus was inactivated during 24 hours' storage at 0° C. in insect juice diluted to $10^{-1.5}$ with 0.85 per cent salt solution.

Experimental evidence was obtained indicating that the virus multiplied at least 100-fold in the insects between the 2nd and 12th day of a 17-day incubation period. The virus reached a high concentration in the insects 6 days before the insects transmitted it to asters. There is some evidence of a decrease in the virus concentration towards the end of the incubation period.

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LITERATURE CITED

1. BAWDEN, F. C. Plant viruses and virus diseases. 272 pp. Chronica Botanica Co., (Leiden). 1939.
2. BENNETT, C. W., and H. E. WALLACE. Relation of the curly-top virus to the vector, *Eutettix tenellus*. Jour. Agr. Res. [U.S.] **56**: 31–51. 1938.
3. BLACK, L. M. Mechanical transmission of aster-yellows virus to leaf hoppers. (Abstract) Phytopath. **30**: 2–3. 1940.
4. FREITAG, J. H. Negative evidence on multiplication of curly-top virus in the beet leafhopper, *Eutettix tenellus*. Hilgardia **10**: 305–342. 1936.
5. FUKUSHI, T. Multiplication of virus in its insect vector. Proc. Imp. Acad. Japan **11**: 301–303. 1935.
6. ———. Retention of virus by its insect vectors through several generations. Proc. Imp. Acad. Japan **15**: 142–145. 1939.
7. HOLMES, F. O. Handbook of phytopathogenic viruses. 221 pp. Burgess Publishing Co. (Minneapolis). 1939.
8. KUNKEL, L. O. Insect transmission of aster yellows. (Abstract) Phytopath. **14**: 54. 1924.
9. ———. Studies on aster yellows. Am. Jour. Bot. **13**: 646–705. 1926.
10. ———. Effect of heat on ability of *Cicadula sexnotata* (Fall.) to transmit aster yellows. Am. Jour. Bot. **24**: 316–327. 1937.
11. ———. Insects in relation to diseases of fruit trees and small fruits. Jour. Econ. Ent. **31**: 20–22. 1938.
12. RAWLINS, T. E., and C. M. TOMPKINS. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. **26**: 578–587. 1936.
13. SEVERIN, H. H. P. Experiments with the aster-yellows virus from several states. Hilgardia **8**: 305–325. 1934.
14. SMITH, R. E. Growing China asters. Hatch Exp. Sta., Mass. Agr. Coll. Bull. 79. 26 pp. 1902.
15. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. VI. The isolation from diseased Turkish tobacco plants of a crystalline protein possessing the properties of tobacco-mosaic virus. Phytopath. **26**: 305–320. 1936.
16. STOREY, H. H. Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. Proc. Roy. Soc. (London), Ser. B, **113**: 463–485. 1933.
17. ———. Transmission of plant viruses by insects. Bot. Rev. **5**: 240–272. 1939.
18. TEN BROECK, C., E. W. HURST, and E. TRAUB. Epidemiology of equine encephalomyelitis in the eastern United States. Jour. Exp. Med. **62**: 677–685. 1935.
19. ZINSSER, H., J. F. ENDERS, and L. D. FOTHERGILL. Immunity: principles and application in medicine and public health. 801 pp. The Macmillan Co. (New York). 1939.

DEVELOPMENT OF SECONDARY TUMORS AND TUMOR STRANDS IN THE CROWN GALL OF SUNFLOWERS

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REVIEW OF LITERATURE

Crown gall, a disease of plants whose causal agent is *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.*, has attracted considerable interest not only because of its practical significance but also because of its alleged similarity to certain neoplastic diseases of animals and man. Smith and his collaborators (9, 10, 12, 13), in a series of papers, stressed these similarities stating that, under certain conditions, strands of tumor tissue may grow, in root-like fashion, from the primary gall into normal tissue. They believed that secondary tumors, which formed at a distance from the primary gall, were always connected with the primary gall by means of a tumor strand. Later, however, Smith (11) modified somewhat his concept of the root-like invasion of normal tissue by the tumor strand and stated that, in some instances at least, it develops by appositional growth. He believed that in such cases a stimulus was transmitted from tumor cells to adjacent healthy cells in one direction only, thereby converting the latter into typical tumor cells. Smith believed these tumor strands to be comparable to certain types of metastases found in malignant tumors of animals and man.

Pelko (5) succeeded in producing tumor strands and secondary tumors in the sunflower by inoculating the developing capitula; thus, he was able to confirm Smith's earlier observation. Küster (3), however, questioned the correctness of Smith's interpretation of the development of secondary galls from root-like tumor strands and stated that conclusive evidence of the infiltration of tumor tissue into normal tissue had not been presented.

Robinson and Walkden (8) hold that in a plant like *Chrysanthemum frutescens* L. secondary tumors are formed only when rudimentary wounded structures, subsequently separated by growth, are infected. They found no evidence of the invasion of tumor growth through normal tissue for any considerable distance. In this plant, the maximum distance that the bacteria extend along the xylem vessels or migrate through the intercellular spaces is never more than a few millimeters. However, in a plant like *Nicotiana affinis* (T. Moore), which has large intercellular spaces, the bacteria were found to migrate in a zoogleal mass for a distance of several inches from the primary gall. According to these workers, the cells lying in the immediate vicinity of the masses of bacteria are stimulated to divide and give rise to tumor strands.

Riker (7) holds that tumor strands and secondary tumors were never found except when inoculations are made into the tops of plants having a number of internodes condensed in the apical bud. When needle-puncture inoculations are made just below the bud of such plants, the injured cells release liquid that forms a continuous channel and serves to distribute the bacteria along a number of nodes and internodes in the condensed bud. As the bud develops and the internodes elongate, the bacteria are distributed for some distance along the stem. Tumor strands are formed by the division of the host cells along the path where the bacteria were distributed at the time of inoculation. According to Riker (7) secondary galls and tumor strands are not secondary from the standpoint of invasion, but result from the inoculation that produces the primary gall. Levine (4) agrees with Riker on this point, but he was not always able to detect a connecting strand of tumor tissue between the primary and secondary galls and, therefore, concluded that the two areas were inoculated at the same time but were subsequently separated by elongating noninoculated tissue. He was unable to verify the work of Robinson and Walkden (8) as regards the presence of bacteria in the deeply staining intercellular spaces of the plants he studied.

Suit and Eardley (15) believe it possible that many of the so-called tumor strands reported by Smith *et al.* to be present in the protoxylem region were internal tumors formed at points where the vessels had ruptured because of the elongation of the stem.

Obviously, there is not complete agreement on certain fundamental points concerning the mechanism involved in tumor-strand and secondary-tumor formation in crown gall. It is the purpose of the present paper, therefore, to review this problem in the light of work that has already been done and to present new evidence that ultimately may aid in its solution.

MATERIALS AND METHODS

Since sunflower plants (*Helianthus* sp. var. Giant Russian) were found to be particularly favorable for the study of the development of secondary tumors and tumor strands, they were used throughout the investigation. The plants were grown in compost soil in 4-in. pots and were kept on a greenhouse bench throughout the course of the experiments.

The culture of *Phytomonas tumefaciens* used for inoculation purposes was the A_6 strain of Riker. The bacteria were introduced by the needle-puncture method. Plants that had reached a height of 10 to 12 inches were used unless otherwise stated. Inoculations were always made at least 3 inches from the apical bud into an internode that had elongated to, or very nearly to, its full length. After inoculation, due care was taken not to injure the plants, since wounds might well serve as avenues for secondary infections and thus lead to confusing results. Care also was exercised in watering the plants, thus preventing spread of the inoculum.

Reisolation of the bacteria was carried out by grinding the desired material together with a few cubic centimeters of nutrient-dextrose broth in a sterile mortar. The thoroughly ground material was permitted to incubate for 4 to 5 hours and then plated out in nutrient-dextrose agar.

In studying the pathological histology, free-hand sections were used throughout. The sections were mounted in lacto phenol (glycerine 40 cc., phenol 20 cc., lactic acid 20 cc.) to which a small amount of safranin was added to aid in the differentiation of certain structures.

EXPERIMENTAL RESULTS

General Appearance of Inoculated Sunflower Plant

Five to 7 days after inoculation with a virulent crown-gall culture, pronounced swellings usually could be noted in the sunflower stems for a distance of several inches above and below the point of inoculation. These swellings, which could often be observed before the primary tumor made its appearance, were usually more pronounced below the point of inoculation than above. During subsequent days, the swellings continued to thicken, and, in extreme cases, a lengthwise splitting of the stem resulted. After a period of 3 to 5 weeks, secondary tumors of two distinct types could be observed on many of the plants. A detailed description of their development is presented in a later section.

Internal Structure of the Swollen Stem

Histological studies of sections of swollen stems and of normal stems revealed several conspicuous tissue changes, the nature and extent of which depended somewhat upon the time that had elapsed following inoculation. Perhaps the most conspicuous changes resulted from cellular disturbances that progressed laterally from the protoxylem region into the pith. Serial sections showed that the disturbances were continuous for a distance of several inches above and below the primary tumor and that they produced strands similar to the tumor strands first described by Smith on the Paris daisy. Often as many as 3 strands were found in a single section.

Another conspicuous cellular change involved the pith cells bordering certain vascular bundles. These cells often showed a pronounced radial elongation. The stimulus that caused this radial elongation appeared to originate in the xylem region of the vascular bundle and to be transmitted laterally toward the center of the pith, usually involving 4 or more successive pith cells. This resulted in a band of radially elongated pith cells extending from the inner edge of a vascular bundle toward the center of the pith. In the more extreme cases, there was also an increase in secondary xylem and this was, in some instances, accompanied by an increase in the width of the medullary rays. The cortical parenchyma appeared unaffected.

The Tumor Strands

Tumor strands may be found within 6 days after the sunflower plants have been inoculated with crown-gall bacteria. In a representative case, a sunflower plant, approximately 13 in. high, was inoculated on December 9 in the first internode above the cotyledonary node. This internode, at the time of inoculation, was $5\frac{1}{4}$ in. long. The inoculation was made 2 in. from the top of the internode and hence $3\frac{1}{4}$ in. from the cotyledonary node below. A uniform swelling of the stem extending throughout the entire internode was noted on December 14, 5 days after inoculation. On December 15, serial sections of the thickened stem were cut and examined and it was found that a fairly well-developed tumor strand was present, which extended continuously a distance of $2\frac{3}{4}$ in. below and $1\frac{1}{4}$ in. above the primary tumor. The internode had not elongated more than $\frac{1}{8}$ in. during the 6-day period. Since numerous other plants treated in the same way showed similar results, it appears evident that rapid elongation of the stem tissue is not a necessary prerequisite to tumor-strand formation in the sunflower.

Tumor strands have been reported present in the cortex of the sweet pea by Riker (7) and in the bark parenchyma of tobacco by Smith (12). They were, however, invariably found in association with the xylem region of the sunflower plant in the present study. At the very earliest stage in the development of the tumor strand, the xylem cells immediately bordering certain of the xylem vessels were stimulated to enlarge, as shown in figure 1a. They continued to enlarge and often elongated in a radial direction, thus causing the edge of the bundle to break loose and be forced into the pith (Figs. 1b and 1c). It would seem from these observations that the strand develops as the result of a stimulus that originates in the xylem vessels and acts in a lateral direction. The mature strand develops as the

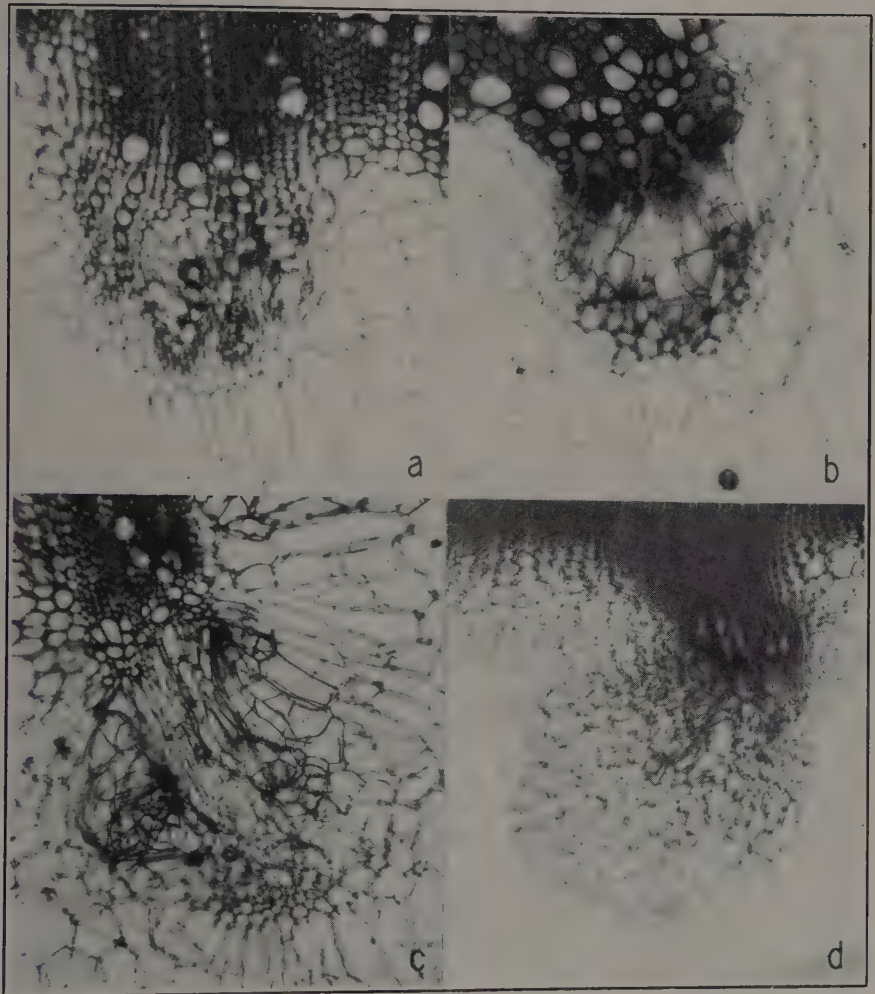

FIG. 1. Cross sections through sunflower stems showing stages in the development of the tumor strand. a. Very early stage showing stimulation of the cells immediately surrounding a xylem vessel. b and c. Later stages. Note the inner edge of the bundle has been forced into the pith by the radially elongating cells. d. Mature strand. a and b, ×85; c, ×52; d, ×42. (Photograph by J. A. Carlile).

result of the repeated division and slight enlargement of the affected cells (Fig. 1d). It is quite obvious that the tumor strand does not develop as a consequence of division in the pith cells in the immediate vicinity of a vascular bundle, as might appear to be the case when only the mature strand is examined, but rather is the result of the enlargement and repeated division of xylem cells immediately surrounding certain of the xylem vessels. It is of interest to note that in many instances the xylem vessels from which the stimulus appeared to arise were yellowish to bright red.

The question as to whether crown-gall bacteria are present in the cells of the tumor strand is difficult to answer. Attempts to isolate them from pieces of strand tissue, carefully dissected out under sterile conditions, have often yielded negative results. Crown-gall bacteria, however, have been isolated in some instances.

Secondary Tumors

Secondary tumors that arise at points distant from the seat of inoculation have been described on a number of plant species by several different investigators. However, as has been pointed out earlier, various interpretations

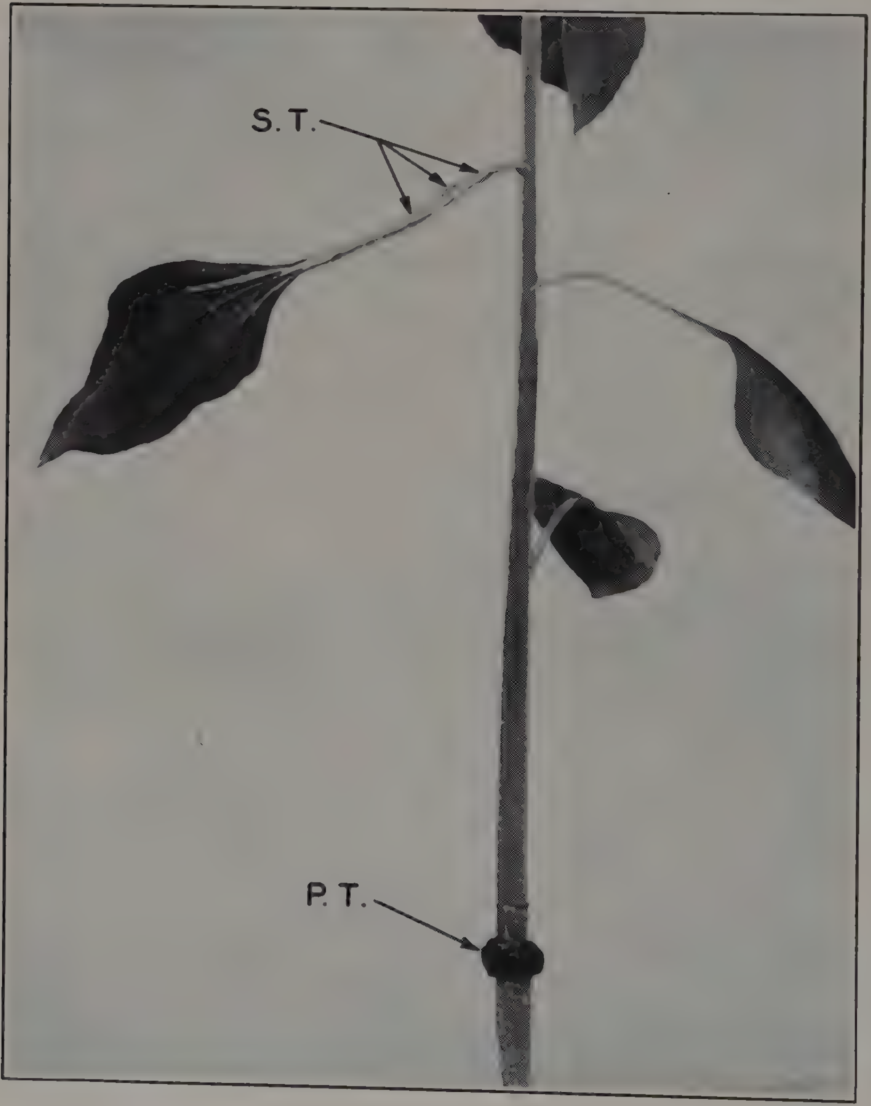

FIG. 2. Young secondary tumors (S.T.) in a petiole located four nodes above the primary tumor (P.T.). The inoculation in this plant was made approximately 4½ inches from the apical bud. Note pronounced tumefaction in one of the veins of the leaf. ×0.55. (Photograph by J. A. Carlile).

as to the mechanism involved in the formation of these structures have been suggested.

In this study on the development of secondary tumors, inoculations were always made at a distance of 4 and 6 inches from the apical bud and usually into the lower portion of an internode that had ceased, or had almost ceased, to elongate. This was done to guard against introducing the bacteria into elongating tissue.

The two distinct types of secondary tumors found to occur on sunflower plants differed both from the standpoint of their relative position on the host plant and, more strikingly, in their internal structure. The first of the two types was found in petioles and appeared to be similar to those described previously by Smith and others on the Paris daisy. About 3 or 4 weeks after inoculation, slight swellings were noted on one or more of the petioles that were frequently located as far as 3–4 nodes above the primary tumor. As the secondary growths developed, the affected petioles began to thicken at certain points and a slight tumefaction often could be noted extending into the larger veins of the leaves (Fig. 2). Often as many as 3 distinct tumors were found in a single petiole.

Histological examination revealed that these structures were outgrowths from tumor strands that usually extended the entire length of the petioles and could frequently be traced into the larger veins of the leaf. These secondary tumors and tumor strands always were associated with the xylem vessels of one or more of the bundles of the leaf trace, and developed in a manner similar to that reported for the tumor strands in a previous section. Here again, the stimulus appeared to originate in certain of the xylem vessels. The cells in the immediate vicinity of the vessels were stimulated to enlarge and, later, divide. As the secondary tumor developed, the overlying tissue became flattened and crushed. Numerous short tracheids were often found and it would appear that they arose through direct transformation of gall tissue. Figure 3 shows an early stage in the development of this type of secondary tumor.

The tumor strand, which was often found to extend the entire length of the petiole and into the midrib and larger veins of the leaf, could not, in most instances, be traced to the primary tumor. Long sections of apparently normal stem tissue were frequently found between the base of the affected petiole and the primary tumor.

It was extremely difficult to isolate bacteria from the secondary tumors and tumor strands found in the petioles. In only a very few instances was it possible to isolate colonies of the crown-gall organism from them.

It seemed conceivable at first, in view of the difficulty of isolating bacteria, that some cell-stimulating substance, which would account for the overgrowths, might be diffusing up and down from the actively growing primary tumor. This possibility was ruled out, however, by the following obesrvation. An attenuated strain of the Chry IIB Stapp culture of *Phytomonas tumefaciens* was isolated, which, when inoculated into sunflower

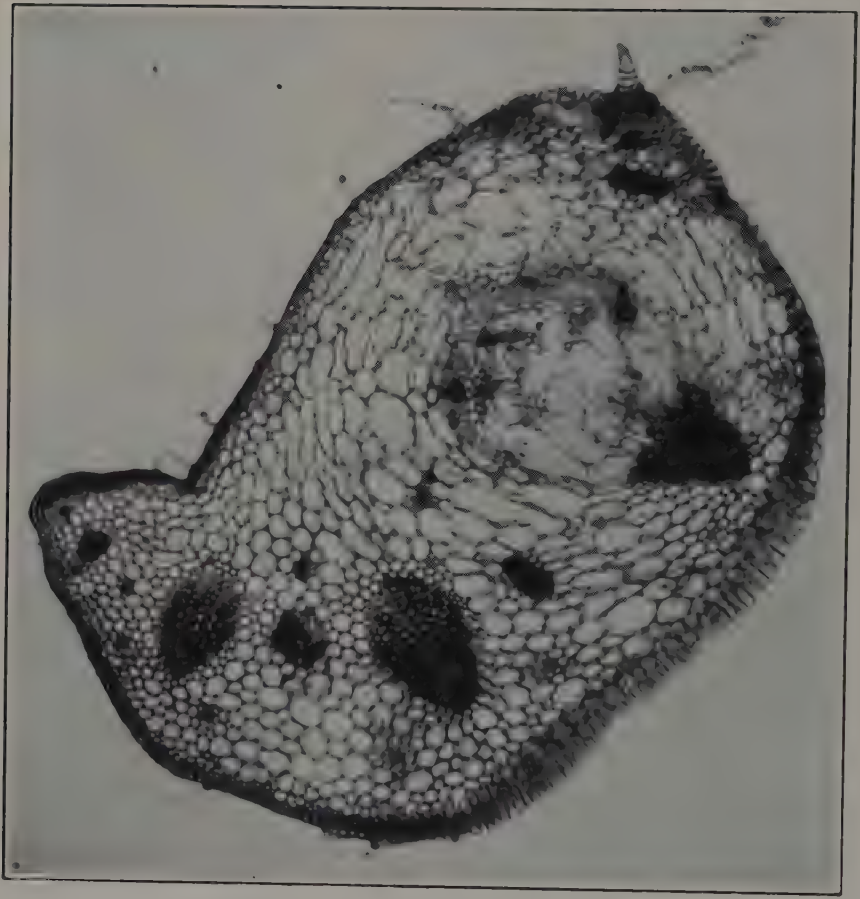

FIG. 3. Cross section through an affected petiole (see figure 2) showing an early stage in the development of a secondary tumor. ×26. (Photograph by J. A. Carlile).

plants, did not produce a primary tumor. Even though no trace of primary-tumor formation was in evidence 4 weeks after inoculation, the sunflower stems had thickened in the characteristic manner and, upon examination, tumor strands were found present. In some instances well-developed secondary tumors also were found (Fig. 4). This proves that the mechanism involved in the formation of these structures does not depend on the formation of a primary tumor. It also demonstrates conclusively that the tumor strands and secondary tumors are not the result of a cell-stimulating substance diffusing from the actively growing primary tumor, since no tumor was present in this instance.

As the sunflower plants develop, it is characteristic for the leaves borne at the lower nodes to die and disappear. After these leaves had fallen off it was not uncommon to find secondary galls at the first and second nodes above the primary tumor (Fig. 5). These secondary growths were of particular interest because of their internal structure. The tumors were char-

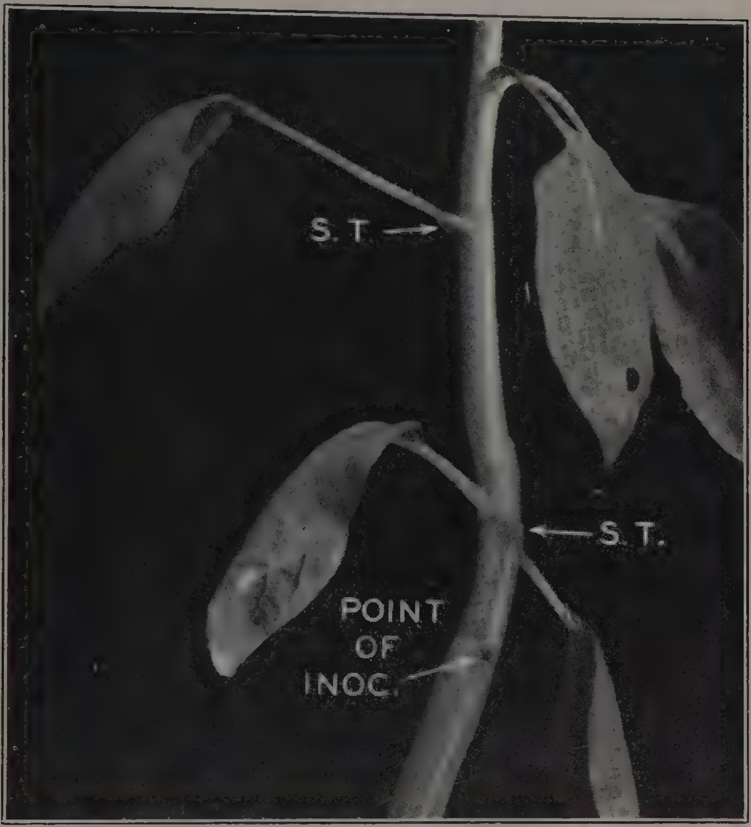

FIG. 4. Well-developed secondary tumors (S.T.) above the point of inoculation (point of inoc.). No primary tumor has formed at the point of inoculation. ×0.94. (Photograph by J. A. Carlile).

acterized by a great increase in secondary xylem, as well as by a well defined, although somewhat enlarged, cortex (Fig. 6). They differed in this way from both the primary and the first type of secondary tumor described previously. The cells of the cortex did not show the pronounced irregularities that characterize typical crown-gall tissue. Their external appearance, however, was similar to that of the other tumors.

An interesting phenomenon was observed on 2 of the several hundred plants that were inoculated during the course of this investigation. Several weeks after inoculation, both plants showed narrow but slightly raised ridges below the primary tumor. The ridges extended continuously from the primary tumor to within a short distance of the soil line, which was slightly more than 6 inches. Seven weeks after inoculation the ridges had grown to about $\frac{1}{8}$ in. above the surrounding stem tissue. The tumor tissue did not break through the epidermis. The stem above the primary tumor appeared normal (Fig. 7).

Histological examinations showed that the ridges consisted essentially

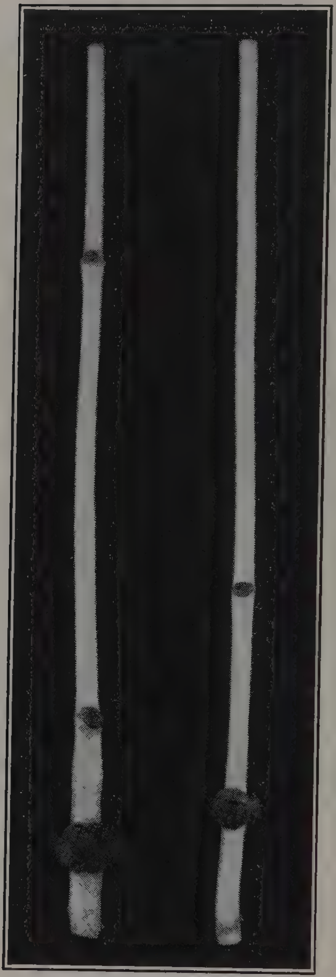

FIG. 5. Secondary galls at first and second nodes above the primary tumor. ×0.56. (Photograph by J. A. Carlile).

of secondary xylem, and that they were very similar in structure to the second type of tumor described in this paper.

Here, again, the formation of these ridge-like overgrowths cannot be explained on the basis of the distribution of the bacteria along the stem due to a rapid elongation of immature tissue, since there was only a slight elongation of the internode into which the inoculum was introduced.

DISCUSSION

There have been two main theories advanced in an attempt to explain the mechanism involved in the formation of secondary tumors and tumor strands in crown gall of plants. Smith believed that these structures resulted as direct outgrowths from the primary tumor and that they were, therefore, comparable to permeation metastases found in human cancers.

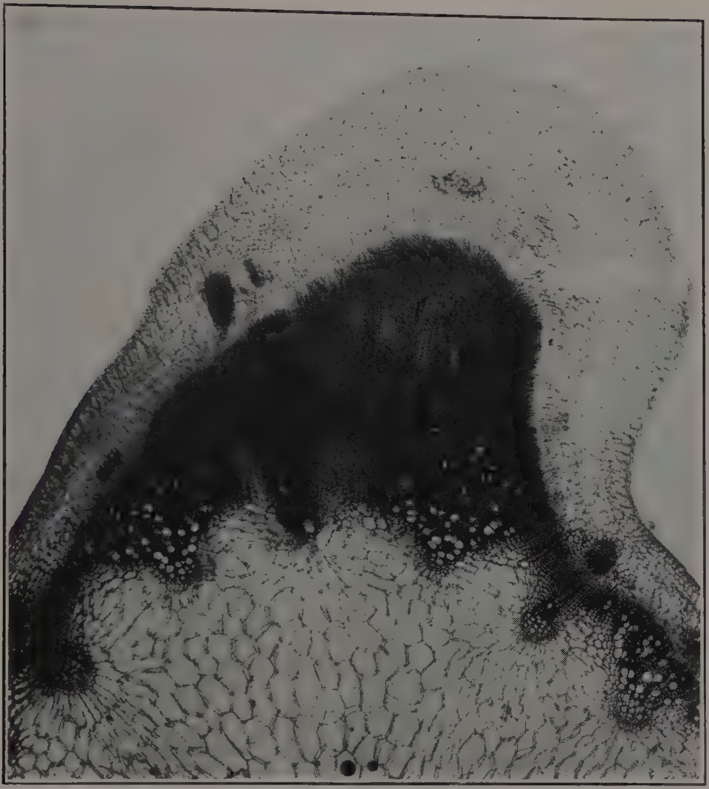

FIG. 6. A cross section through a secondary gall such as is shown in figure 5. Note great increase in secondary xylem and the well defined although somewhat enlarged cortex. × 16.8. (Photograph by J. A. Carlile).

Although the writer believes that the secondary tumors and tumor strands found on sunflowers are comparable in every respect to those described by Smith *et al.* on the Paris daisy, he is not in full agreement with Smith's conclusions. It seems quite clear from figure 1 that the tumor strand is not pushing down from the primary tumor in root-like fashion, as suggested by Smith, but rather that its initial development begins in the xylem region of the vascular bundle and that, as it develops, it expands laterally into the pith. The fact that the inner edge of a fibrovascular bundle may be torn loose and be forced into the pith by radially elongating cells of the developing tumor strand serves to substantiate this contention.

If the strand were pushing up or down from the primary tumor in a root-like fashion, one would expect to find a strand tip that might be compared to the tip of a developing rootlet. However, it was found that the strands merely became smaller as the distance from the primary tumors increased and that they gradually disappeared entirely. The pith cells bordering the affected bundles were often found to have elongated radially for some distance beyond the last trace of the tumor strand. The fact that secondary

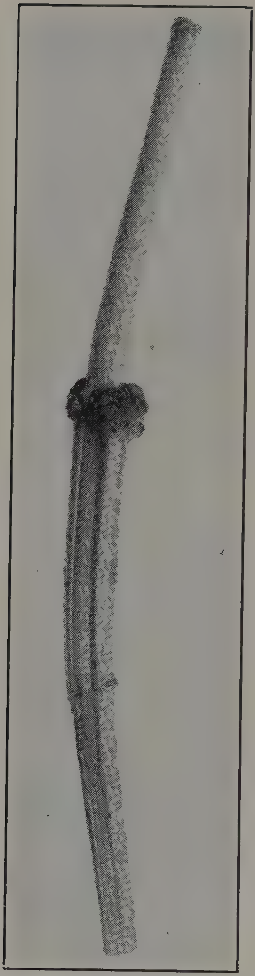

FIG. 7. Ridge-like overgrowths below the primary tumor. Note stem above the primary tumor appears normal. × 0.73. (Photograph by J. A. Carlile).

tumors are often separated from the primary tumor by apparently normal stem tissue would certainly argue against Smith's contention that the former are a direct outgrowth of the primary tumor. This fact, which was pointed out previously by Levine (4), has been substantiated in the present investigation.

As was stated above, Riker (7), Levine (4) and others have suggested a second explanation regarding the development of strands and secondary tumors. These investigators contend that secondary tumors and tumor strands are formed only when inoculations are made into, or very close behind, the terminal bud. Riker states that when cells are injured during the process of inoculation, liquid is released that serves to distribute the bacteria through a number of nodes and internodes in the condensed bud.

Attempts have been made in this paper to show that it is possible to secure both tumor strands and secondary tumors when inoculations are made at some distance from the apical bud into tissue that had elongated to, or nearly to, its full length. Since the liquid that was released during inoculation extended in most instances only a comparatively short distance from the point of inoculation, it could not account for the distribution of the bacteria and subsequent formation of such tumor strands and secondary tumors as are reported here.

While the writer does not question the fact that Riker (7), Levine (4), and others have been able to secure strands and secondary tumors by inoculating immature tissue, he merely wishes to emphasize the fact that it is possible to secure them in sunflowers by inoculating internodes that had elongated to, or nearly to, their full length.

The possibility that the secondary growths were due to bacteria in the adjacent xylem vessels has to be considered. Several investigators have demonstrated the presence of the crown gall bacteria in the xylem vessels of various plants. Robinson and Walkden (8) observed them in the vessels of *Chrysanthemum frutescens* and *Nicotiana affinis* and believed them capable, under certain conditions, of causing disturbances in the vicinity of the invaded vessels. Although the bacteria were not found to migrate more than 2 mm. in the xylem vessels of Chrysanthemum, they were believed to be carried passively in elongating tissue when inoculations were made into the apical bud. Secondary tumors arose when the needle penetrated a vessel in the rudimentary leaf. It was believed that the bacteria were restricted to the xylem vessels in the secondary tumors of this plant.

Riker (6) found no evidence that the bacteria could produce galls if confined to the vascular elements of tomato; he was, however, able to show that if they were provided with an avenue of escape, they could stimulate gall production. Suit and Eardley (15) are in general agreement with Riker in this belief. These workers, however, described a type of extruding tumor that, according to them, was a modified rootlet. The formation of this type of tumor was stimulated by the presence of bacteria in the mechanically plugged or imperfect vessels.

Several other workers (1, 2) also have found crown-gall bacteria present in the xylem vessels, but made no mention of cellular disturbances in the vicinity of the vessels. Stapp, Müller, and Dame (14) were able to isolate *Phytomonas tumefaciens* from apparently healthy tissue at a distance of over 100 cm. above and below the primary tumor in *Datura tatula.*

While one may not feel fully confident of the exact course followed by the bacteria in stimulating tumor strand and secondary-tumor formation in the present study, the evidence available seems to indicate that they are capable of traveling through the xylem vessels of the sunflower plant for considerable distances. As has been pointed out earlier, the tumor strands and secondary tumors always appeared to originate in association with the xylem region of the vascular bundle and more particularly in association with certain of the xylem vessels. The vessels from which the stimulus appeared to arise were often discolored, and this led to the belief that the bacteria were present in them. Whether the bacteria remain in the vessels while stimulating the neighboring host cells to enlarge and divide is not known with certainty. Since it has been shown, however, that secondary tumors and tumor strands may be produced by an attenuated strain of *Phytomonas tumefaciens,* even though no primary tumor be formed, it appears likely that the mechanism involved in the formation of these structures in sunflower plants is essentially different from that concerned in the formation of the primary tumor. The pronounced differences in the type of cell stimulation found in the tumor strand as compared with that found in the primary tumor is further evidence in support of this view. It is believed probable, therefore, that the bacteria remain confined to the vessels and that under their influence, cell-stimulating substances are formed that diffuse laterally and bring about cellular disturbances in adjacent tissues. The same substance or substances responsible for tumor-strand formation are probably also active in stimulating the pith cells to elongate radially.

SUMMARY

The problem under consideration is concerned chiefly with the development of secondary tumors and tumor strands in sunflower plants affected with crown gall.

It has been shown that these structures can be produced by introducing the crown-gall bacteria at a distance of 4 to 6 inches from the apical bud into internodes that had elongated to, or almost to, their full length. This proves that these structures are truly secondary and do not depend for their development upon the rapid elongation of immature tissue. The ridge-like overgrowths, which sometimes extend from the primary tumor to within a short distance of the soil line, are also secondary.

Tumor strands and secondary tumors were invariably found in association with the xylem region of sunflower plants. The strands are not direct outgrowths of the primary tumor, as suggested by Smith, but develop laterally from the xylem region into the pith.

Two distinct types of secondary tumors are described. They differ both from the standpoint of their relative positions on the host plant and, more strikingly, in their internal structure.

It has been shown that secondary tumors and tumor strands may be produced by an attenuated strain of *Phytomonas tumefaciens,* even though no primary tumor is formed. This suggests that the mechanism involved in the formation of these secondary structures may not be identical with that concerned in the formation of the primary tumor.

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LITERATURE CITED

1. HILL, J. B. The migration of *Bacterium tumefaciens* in the tissue of tomato plants. Phytopath. **18**: 553–564. 1928.
2. ————, W. H. BRITTINGHAM, F. P. GIBBONS, and G. W. WATTS. Further notes on *Bacterium tumefaciens* and its host relationships. Phytopath. **20**: 179–186. 1930.
3. KÜSTER, E. Über die Gallen der Pflanzen (neue Resultate und Streitfragen der allgemeinen Cecidologie). Fortschr. d. Naturw. Forsch. **8**: 115–160. 1913.
4. LEVINE, M. The so-called strands and secondary tumors in the crown gall tissue. Phytopath. **15**: 435–451. 1925.
5. PELKO, J. Über die Smith'schen Rübentumoren. Zeitsch. für Zuckerindus. Böhmen. **39**: 204–219. 1915.
6. RIKER, A. J. Some relations of the crown gall organism to its host tissue. Jour. Agr. Res. [U.S.] **25**: 119–132. 1923.
7. ————. Some morphological responses of the host tissue to the crown gall organism. Jour. Agr. Res. [U.S.] **26**: 425–435. 1923.
8. ROBINSON, W., and H. WALKDEN. A critical study of crown gall. Ann. Bot. **37**: 299–324. 1923.
9. SMITH, E. F. Further evidence that crown gall of plants is cancer. Science (n.s) **43**: 871–889. 1916.
10. ————. Studies on crown gall of plants: Its relation to human cancer. Jour. Cancer Res. **1**: 231–309. 1916.
11. ————. Appositional growth in crown gall tumors and in cancers. Jour. Cancer Res. **7**: 1–49. 1922.
12. ————, N. A. BROWN, and L. MCCULLOCH. The structure and development of crown gall. U. S. Dept. Agr. Bur. Plant Indus. Bull. 255. Pp. 60. 1912.
13. ————, ————, and C. O. TOWNSEND. Crown gall of plants: Its cause and remedy. U. S. Dept. Agr. Bur. Plant Indus. Bull. 213. Pp. 215. 1911.
14. STAPP, C., HEINR. MÜLLER, and F. DAME. Der Pflanzenkrebs und sein Erreger *Pseudomonas tumefaciens.* VII Mitteilung: Untersuchungen über die Möglichkeit einer wirksamen Bekämpfung an Kernobsthölzen. Zentralbt. f. Bakt. II. **99**: 210–276. 1938.
15. SUIT, R. F., and E. A. EARDLEY. Secondary tumor formation on herbaceous hosts induced by *Pseudomonas tumefaciens* (Sm. and Towns.). Scient. Agr. **15**: 345–357. 1935.

QUANTITATIVE COMPARISON OF METHODS FOR STERILIZING SOLUTIONS OF ORGANIC COMPOUNDS USED IN CULTURE MEDIA[1]

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An important phase of the investigations by the authors on the chemical nature of resistance in plants to *Phymatotrichum omnivorum* (Shear) Duggar consists in evaluating the fungicidal activity of various organic compounds. Some of these compounds have been isolated from plants whose resistance is being investigated, while others are known either to be present in plants or related to those present. A method of testing in liquid culture was chosen as offering greatest promise in indicating the relative toxicity of compounds without an attempt to duplicate actual plant conditions. The paucity of literature regarding the merits of various methods of sterilization made necessary an investigation to develop a technique whereby chemicals could be incorporated into sterile liquid media at a definite concentration with a minimum of loss or alteration, either of the compound or of the substrata.

[1] Approved by the Director as Contribution No. 601, Technical Series, of the Texas Agricultural Experiment Station.

Various sterilization procedures have been used in testing the laboratory effects of chemicals on microorganisms. When tests are conducted for short periods only, as during germination of spores, possible fungous or bacterial contamination carried by the chemical itself is not likely to interfere with the results. For this reason most investigators who have tested fungicidal compounds in liquid culture and who have used organisms that either grew very rapidly or tolerated high concentrations of the fungicide have found it sufficient to make up the chemical in water and add the required amount to the sterile medium. This procedure is inapplicable, however, when the tests extend for a long time and the solution of the compound added is so dilute as not to be self-sterile. Difficulty with these methods is encountered also with substances which are sparingly soluble in water. Filtration through Berkefeld or other suitable filter has been used to avoid possibility of decomposition by heat. But, because of the time and equipment required and the uncertainty of complete sterilization (11), it becomes a burdensome task when there are several solutions to be tested. Another procedure, sometimes used, consists in weighing the required amount of chemical into a dry flask, autoclaving, and then adding sterile medium to the flasks. This is time-consuming, particularly with liquids, since they are difficult to weigh and transfer in small quantities. Moreover, the more volatile ones are apt to be lost unless the flask is tightly stoppered. Neither method seemed applicable to the present problem; therefore, further consideration of the matter was abandoned. Probably the most commonly practiced method of sterilization consists in adding the compound to the medium in the required concentration and autoclaving for 15 to 20 min. at a pressure of 15 lb. Where hydrolysis or decomposition is anticipated, sterilization by steaming at 100° C. on successive days has been used instead of heating under pressure.

In spite of well recognized disadvantages of autoclaving, such as the possibility of hydrolysis or other decomposition of sugars, starches, glucosides, and proteins, this procedure has been used by several investigators in preparation of plant extracts for testing fungous responses. It might be expected, moreover, that many oily compounds, such as essential oils and fatty acids, would be at least partly lost by volatilization. Apparent loss of potent fractions from onion juices on heating was recognized by Walker, Lindegren, and Bachmann (14) and by Brown (1). Walker and Link (15), however, in a subsequent investigation on the toxicity of phenols to fungi apparently autoclaved the compounds in presence of the culture medium. There seem to be no quantitative studies on losses of known compounds while autoclaving.

The authors observed extensive losses of organic compounds by volatilization during steam sterilization. Consequently in previous investigations (3, 4, 9) involving the fungicidal testing of compounds, the chemical was dissolved in 95 per cent alcohol and the desired quantity (usually not more than 1 ml.) added to each dry sterile flask by means of a micropipette. The flask was then allowed to stand at room temperature until the alcohol had completely evaporated through the plug (1–2 days), leaving the sterile compound in the flask. Sterile medium was then added quantitatively. For such aseptic addition of medium, the automatic zero pipette described elsewhere was rapid and accurate (10). The alcohol technique also is applicable to the sterilization of mixtures, such as alcoholic solutions of plant extracts. Substances less soluble in alcohol, such as sugars and glucosides, can be sterilized by weighing the dry sample into a sterile flask, adding sufficient alcohol to wet the sample and allowing the alcohol to evaporate. This alcohol-sterilization method possesses the great advantage of no possibility of alteration from heating, and is satisfactory for most compounds. Liquids with appreciable vapor pressure at room temperature are, however, inclined to volatilize with the alcohol and thus be lost. In a few cases there seems to be creeping of solid compounds into the neck of the flask, possibly by a process of sublimation. Where loss occurs by volatilization, it was found advisable to pipette 0.5 ml. or less of 50 per cent alcoholic or 0.25 ml. or less of 95 per cent alcoholic solution into the sterile flask either before or after the sterile medium was added. In this way there was no loss by evaporation. The disadvantages of this procedure are that the smaller quantities are less readily measured with the same precision and rapidity as are larger quantities, and that, under certain conditions, the presence of alcohol might be objectionable. Satisfactory sterilization was obtained with the described alcohol method. The number of contaminations resulting from the entire technique never exceeded 5 per cent.

In this investigation the losses by autoclaving, by intermittent steaming, and by use of the original alcohol-evaporation technique are compared, using representative organic compounds. In testing the autoclaving procedure, the compounds were added to water and to 2 liquid media used for culture of fungi, Czapek[2] and No. 70,[3] in order to observe the effect of the nature of the solution on the amount of loss. From these tests it should be possible to estimate the conditions under which each sterilization procedure is applicable and to formulate general rules for the sterilization of various types of compounds.

METHODS

The compounds used are representative of the various types of organic molecules that have been encountered either as fungicides or as plant constituents. The list contains examples of compounds that are varied in characteristics with respect to chemical classification, stability, vapor pressure, solubility, and state of aggregation. The concentration was in all cases 0.01 M, since this strength was one which could conveniently be determined with accuracy and was one extensively encountered. The samples were obtained by adding 1 ml. of stock solution in alcohol to 50 ml. of water or culture medium contained in a 250-ml. Florence flask, when heat sterilization was used, or to the dry flask when the alcohol was allowed to evaporate. The flasks were closed with cotton plugs before treatment. Four samples of each compound were measured into flasks; two of these were titrated to determine the exact initial amount present and two were titrated after appropriate sterilization. The difference between these two figures was computed as loss, and the percentage of loss was reported.

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[2] Czapek's nutrient solution consisted of: $MgSO_4$, 0.5 g.; KH_2PO_4, 1.0 g.; KCl, 0.5 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; asparagin, 1.0 g.; dextrose, 30 g.; water 1000 ml.

[3] Modified nutrient solution No. 70 consisted of: $MgSO_4$, 0.75 g.; K_2HPO_4, 1.35 g.; NH_4NO_3, 1.00 g.; KCl, 0.15 g.; and dextrose, 40.00 g. per liter. After removal of heavy metals by the method of Steinberg, Mn^{++}, Cu^{++}, Fe^{++}, and Zn^{++} were added at concentrations of 2.5 p.p.m.

The acids were determined by titration with 0.05 N sodium hydroxide using phenolphthalein indicator. The following compounds were assayed by means of bromide-bromate solution as described by Kolthoff and Furman (6): aniline, phenol, resorcinol, thymol, 8-hydroxyquinoline, and m-cresol. It also was found possible to estimate anisole and resorcinol dimethyl ether by the same method; each molecule of these compounds reacted with two molecules of bromine. The o-hydroxybenzyl alcohol liberated by hydrolysis of salicin was estimated by bromide-bromate titration after extracting the acidified aqueous solution with ether and then removing the phenol from the ether with 5 per cent sodium hydroxide. The estimation of quinoline was accomplished by precipitation from an acidified aqueous solution with saturated picric acid in water, allowed to stand in the refrigerator, filtered, washed with cold saturated solution of quinoline picrate and the dry precipitate weighed. Vanillin, p-hydroxybenzaldehyde, and benzaldehyde, including that from amygdalin, were determined gravimetrically by precipitation with 2, 4-dinitrophenylhydrazine, according to Iddles, Low, Rosen, and Hart (5).

The Czapek medium was prepared according to Walker and Link (15) and had a pH value of 5.43; the medium No. 70 was prepared as previously reported (3, 4) and had a pH value of 6.51. The 15-min. period of autoclaving was accurately regulated, since the amount of loss varied with the length of the heating period. The thermostat in the exhaust port of the autoclave was set to maintain a temperature of 120° C. Three consecutive periods of 30 minutes each in the Arnold sterilizer were used for the flasks treated by intermittent steaming at 100° C. The period necessary in this type of sterilization is variously given from 15 minutes to one hour. In this experiment 30-minute periods were chosen as an intermediate value that would give an estimate of the loss to be expected from this type of treatment. The positions of the flasks in the sterilizer were changed after each period of steaming to insure greater uniformity of treatment.

RESULTS

The losses resulting from the various methods of sterilization are shown in table 1. Those incurred on heating solutions of the fatty acids gradually increased as the length of carbon chain increased from 1 to 9; thereafter, they decreased. When the number of carbon atoms was 10 or less, the losses by autoclaving in water were less than those resulting from intermittent steaming in water or from autoclaving in Czapek's solution and in medium No. 70.

However, when the number of carbon atoms was more than 10, the losses by autoclaving in water were greater than those from the other 3 procedures. Among the fatty acids up through C_{10} the losses in Czapek's medium are slightly greater than in Medium No. 70. There seems to be little regularity among the aromatic compounds tested; although, in general, the loss from water was less than from the two salt solutions.

TABLE 1.—*Losses of compounds during sterilization*

Compound	Evaporation of 95% alcohol	Intermittent steaming in water	Autoclaving in following media:		
			Water	Medium No. 70	Czapek's solution
Aliphatic Acids	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Formic	72.4	2.3	1.2	18.3	25.4
Acetic	46.5	5.4	4.5	20.5	26.3
Propionic	16.1	9.2	6.2	26.3	30.7
Butyric	6.0	13.5	9.2	29.6	32.8
Valeric	1.7	18.2	14.4	30.7	33.2
Caproic	1.6	24.7	23.5	35.8	40.4
Heptylic	0.7	32.0	29.8	42.6	46.4
Caprylic	0.0	46.5	36.1	52.8	57.1
Pelargonic	0.0	50.2	48.5	70.2	76.8
Capric	0.0	22.6	38.6	60.6	62.5
Undecylic	0.0	11.5	19.0	16.0	8.8
Lauric	0.0	5.8	11.2	7.9	7.3
Tridecylic	0.0	4.6	9.0	8.7	4.0
Oleic	0.0	1.6	1.3	0.0	0.0
Aromatic Acids					
Benzoic	0.0	3.2	3.6	13.4	22.3
m-Toluic	0.0	4.6	4.2	20.9	32.6
Phenols					
Phenol	1.4		10.0	16.1	15.1
Anisole	89.0		96.7	100.0	100.0
m-Cresol	1.5		14.3	21.8	20.5
Resorcinol	0.0		0.0	3.4	1.7
Resorcinol dimethyl ether	2.9		77.2	71.9	73.8
Hydroquinone	0.0		0.0	1.0	0.0
Thymol	5.9		49.8	63.8	65.2
Amines					
Aniline	8.9		24.8	31.1	30.8
Quinoline	1.3		33.0	28.1	29.4
8-Hydroxyquinoline	0.0		19.4	21.5	19.8
Aldehydes					
Benzaldehyde	22.8		68.1	62.5	49.3
p-Hydroxybenzaldehyde	0.0		0.0	0.0	0.0
Vanillin	0.0		0.2	0.0	0.0
Esters					
Methyl salicylate	0.0		74.2	59.2	51.1
Amides					
Pelargonamide	0.0		1.0	4.2	4.0
Glucosides					
Salicin	0.0		0.0	0.0	0.0
Amygdalin	0.0		0.0	0.0	0.0

As shown in the table, all fatty acids through tridecylic show appreciable volatilization upon heating. Benzoic and toluic acids also are lost, particularly from nutrient solutions. Less soluble and more volatile substances like the ethers, anisole, and dimethoxybenzene, benzaldehyde, and methyl salicylate are almost completely distilled. On the other hand, the more water-soluble, less volatile substances, like hydroquinone, resorcinol, p-hydroxybenzaldehyde, and vanillin suffer only slight loss, if any.

Under the conditions used, no appreciable oxidation of aldehydes, such as benzaldehyde, p-hydroxybenzaldehyde, and vanillin, to the corresponding acid was found. Hydroquinone seems to be oxidized to a slight extent to quinone in medium No. 70, which is less acid than the other media. This is in harmony with the observation that hydroquinone is more readily oxidized by atmospheric oxygen in an alkaline medium. The lack of oxidation, even at high temperature, may be attributed either to removal of the air from the flask by the incoming steam before oxidation has opportunity to occur or to the resistance of the compound to oxidation at the temperature and pH of the solution used. There was no detectable hydrolysis during autoclaving of any of the solutions of the ester methyl salicylate or of the glucosides amygdalin and salicin, as measured by titration of their respective hydrolytic products. There was slight hydrolysis of the amide, pelargonamide, in the salt solutions and in water (4 and 1 per cent, respectively). Since all the solutions tested were nearly neutral, the lack of hydrolysis of these compounds was not unexpected, although in more acid media, such as are sometimes used for culture of fungi, the degree of hydrolysis would be expected to increase.

When the original alcohol-evaporation technique was used, the losses in the fatty-acid series decreased as the number of carbon atoms increased. The losses were very great with formic and acetic, slight with valeric, caproic and heptylic, and zero with caprylic and all others. Anisole, benzaldehyde, aniline, and thymol lost more than 5 per cent; all other losses of aromatic compounds were slight or zero. When the original alcohol technique was modified, and the alcoholic solution was added directly to the nutrient solution, there was obviously no opportunity for loss.

FACTORS INFLUENCING LOSS OF SOLUTE

The heating of a solution in a steam sterilizer resembles conventional steam distillation in that the loss depends upon the vapor pressure of the compound in presence of water. It differs from steam distillation in that there is no loss of water from the flask, since the vapor pressure of water on the outside is equal to or greater than that on the inside. An excellent discussion of the principles involved in steam distillation is given by Morton (7). Since the original concentration, temperature, and time of heating are the same for all compounds, the relative loss by volatilization of each should be proportional to its vapor pressure in contact with water at 100° or 120° C. respectively, when the flask is heated. As Morton points out, compounds that are insoluble have vapor pressures not substantially altered by the presence of water. For soluble compounds, the vapor pressure is given by Henry's law, which states that the vapor pressure of the solute above a solution equals the product of the vapor pressure of the pure solute times its mol fraction in the solution. This relation, however, holds only for ideal solutions and cannot be applied to solutions in water of such polar molecules as acids, amides, alcohol, phenols, and amines, so that the amount of loss of these substances is not necessarily proportional to the vapor pressure and mol fraction of the pure solute. For example, the vapor pressures at 120° C. of quinoline, m-cresol, aniline, and phenol are 21, 49, 97, and 100 mm., respectively; the losses on autoclaving in water are 33, 14, 25, and 10 per cent. The abnormal volatility of these compounds has been related to degree of hydration (13) and of electrolytic dissociation (8). Even though the exact reason for divergence from ideal solutions is not known, it is nevertheless possible to observe certain relations between the structure of compounds and their volatility. Compounds with polar groups are more soluble and less volatile than those without groups that can be hydrated. As the relative proportion of these groups per molecule decreases, volatility increases. For example, in Czapek's solution these losses were found: benzoic acid 22.3, m-toluic acid 32.6, phenol 15.1, m-cresol 20.5, thymol 65.2 per cent, respectively. Dyer (2) found that in steam distillation, with the volume of distilling liquid kept constant, the percentage of fatty acid distilled in a given quantity of water was proportional to the length of the carbon chain. He did not investigate acids with more than 9 carbon atoms. The losses from steam sterilization are likewise proportional to the length of carbon chain through C_9 because of decreasing solubility. After that the vapor pressure becomes so small because of high molecular weight that the losses diminish. When the number of polar groups in a molecule increases, the volatility falls off: phenol is more volatile than hydroquinone and resorcinol; benzaldehyde is more volatile than p-hydroxybenzaldehyde. When the effect of polar groups is altered by esterification or etherification, the loss is greatly increased: for example, methyl salicylate is more volatile than salicylic acid; anisole more than phenol; resorcinol dimethyl ether more than resorcinol.

The volatilities of certain isomeric compounds are influenced by the position of the substituents, whether ortho, meta, or para. Sidgwick (12) has found that ortho compounds often have the ability to form chelate rings, which lowers their ability to associate either with water or with themselves, and increases their volatility. In this investigation o-toluic acid was found to be more volatile than m- or p-toluic acids, as would be expected from Sidgwick's data.

Virtanen and Pulkki (13) have derived an equation from which they obtained a constant k to express the relative volatility of compounds with steam. In table 2 are arranged a number of compounds for which both the k value (taken from the article by Virtanen and Pulkki) and the loss upon sterilization are given. There is close agreement between the k value and the amount of loss.

In most cases the amount of loss from water was different from that with the two salt-containing media. This may have been a combination of the

TABLE 2.—*Comparison of the volatility of organic compounds in steam distillation with the losses on steam sterilization*

Compound	Constant *k* of Virtanen and Pulkki	Loss on autoclaving	Loss on intermittent steaming
Benzoic acid	0.270	3.6	3.2
Formic acid	0.370	1.2	2.3
m-Toluic acid	0.420	4.2	4.6
Acetic acid	0.657	4.5	5.4
Propionic acid	1.24	6.2	9.2
Phenol	1.94	10.0	....
Butyric acid	1.96	9.2	13.5
Aniline	5.51	24.8	....
Thymol	12.0	49.8	....
Benzaldehyde	18.0	68.1	....

factors of hydrogen-ion and of salt concentration. Czapek's medium, which was more acid than medium No. 70, caused greater losses with the more soluble acids; and it is possible that the decreased solubility of fatty acids in the more acid solution was a factor in this loss. Virtanen and Pulkki (13) have found that the addition of salt brings about a very great change in volatility and that all salts do not behave similarly, as some are more effective than others in increasing volatility. Also it was found that certain acids were affected more by the presence of salt than others. A similar variation in loss was found during autoclaving.

Since the flasks are plugged with cotton during the sterilizing period, the phenomenon of diffusion may play a rôle in the losses both from heat sterilization and from the alcohol treatment. The interstices between the cotton fibers are, however, so large compared with the size of molecules that the plug is permeable to all molecules. To test the effect of the cotton plug on amount of loss on heating, duplicate sets of flasks, with and without plugs, were autoclaved for 15 minutes at 15 pounds' pressure and the losses determined. The ratio: loss without plug/loss with plug for the acids tested were as follows: acetic 1.16, propionic 1.22, butyric 1.30, valeric 1.29, caproic 1.32, heptylic 1.28, caprylic 1.37, pelargonic 1.25, capric 1.24, undecylic 1.05, lauric 1.29, tridecylic 1.15, benzoic 1.25, o-toluic 1.38, m-toluic 1.35, p-toluic 1.76, average 1.29. These ratios appear to fluctuate around a mean of 1.3 and are not noticeably affected by the molecular weight. The first value and the last 5 are probably less exact than the others because the differences on which they were based were very small.

An evaluation of the factors involved in the loss of solute in presence of evaporating alcohol is very difficult, since the vapor pressures of most organic compounds are very slight at room temperatures and are not known in presence of alcohol. As would be expected the relationships are very different from those in water because of different solubility properties. The only compounds lost are those that are appreciably volatile; and it is difficult to say whether more or less would be lost if they were present alone in the flask. On the other hand, some compounds having marked affinity for water seem less volatile than would be expected because they remain in solution with the water from the 95 per cent alcohol. In general, this method is satisfactory with solids and liquids of high boiling point; with more volatile liquids a preliminary test should be made with this as with any other method to determine whether the amount of loss is such as to make a modification advisable.

SUMMARY AND CONCLUSIONS

The losses from a number of typical organic compounds at 0.01 M concentration were compared by the following methods of sterilization: (1) by autoclaving in presence of water, medium No. 70, and Czapek's solution, (2) by intermittent steaming in presence of water, and (3) by alcohol. With the series of fatty acids, the losses incurred by heat treatment increased progressively from formic to pelargonic and then decreased. For acids with fewer carbon atoms than 10, the losses were greater in the salt solutions used as media than in water; for most of the other compounds tested the differences were slight. There is close agreement between the volatility of compounds in steam distillation reported by others and the amount of loss on steam sterilization. The more water-soluble and strongly polar compounds (alcohols, phenols, acids, amides, amines) are vaporized to a smaller extent than the more volatile, less soluble and less strongly polar compounds (esters, ethers, aldehydes, hydrocarbons). The amount lost, when the flasks are autoclaved without cotton plugs, is about 1.3 times that lost with plugs. Of all the 33 compounds tested, only formic, acetic, and propionic acids suffered greater losses by sterilization with the original alcohol evaporation technique than by heat treatment. The data seem to warrant the following conclusions regarding the applicability of the various methods tested:

1. Autoclaving of most liquids, particularly the less soluble, more volatile ones, gives rise to appreciable losses. The amount of volatilization from culture media containing salt is usually greater than that from water alone. The losses from intermittent steaming may be even more pronounced than those from autoclaving, depending upon the temperature and heating period in each case.

2. There is usually little loss by vaporization of solids, particularly the more soluble and less volatile ones.

3. There is no appreciable hydrolysis of esters, glucosides, and amides, nor oxidation of aldehydes and hydroquinone in approximately neutral solution.

4. Except for the most volatile compounds, losses by the alcohol-sterilization technique given are negligible. Where such losses do occur, they can be avoided by adding concentrated solutions in 50 to 95 per cent alcohol directly to the medium.

5. When a great degree of accuracy is required one should test the contemplated method of sterilization to determine whether it is applicable to the types of compounds under consideration.

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LITERATURE CITED

1. BROWN, W. Studies in the physiology of parasitism. IX. The effect on the germination of fungal spores of volatile substances arising from plant tissues. Ann. Bot. **36**: 285–300. 1922.
2. DYER, D. C. A new method of steam distillation for the determination of the volatile fatty acids, including a series of colorimetric qualitative reactions for their identification. Jour. Biol. Chem. **28**: 445–473. 1917.
3. GREATHOUSE, GLENN A., and G. M. WATKINS. Berberine as a factor in the resistance of *Mahonia trifoliolata* and *M. Swaseyi* to Phymatotrichum root rot. Amer. Jour. Bot. **25**: 743–748. 1938.
4. ————, and NEIL E. RIGLER. The chemistry of resistance of plants to Phymatotrichum root rot. IV. Toxicity of phenolic and related compounds. Amer. Jour. Bot. **27**: 99–108. 1940.
5. IDDLES, H. A., A. W. LOW, B. D. ROSEN, and R. T. HART. Determination of carbonyl compounds by means of 2, 4-dinitrophenylhydrazine. Ind. and Eng. Chem. (Anal. Ed.) **31**: 102–103. 1939.
6. KOLTHOFF, I. M., and N. H. FURMAN. Volume II. Practical Volumetric Analysis. John Wiley and Sons. (New York.) 1929.
7. MORTON, A. A. Laboratory technique in organic chemistry. McGraw-Hill Co. (New York.) 1938.
8. REILLY, J., and W. J. HICKINBOTTOM. The influence of electrolytic dissociations on the distillation in steam of the volatile fatty acids. Sci. Proc. Dublin Soc. **16**: 120–130. 1920.
9. RIGLER, NEIL E., and GLENN A. GREATHOUSE. The chemistry of resistance of plants to Phymatotrichum root rot. VI. Fungicidal properties of fatty acids. Amer. Jour. Bot. **27**: 99–108. 1940.
10. ————, and ————. An automatic zero pipette for dispensing sterile culture media. Science **92**: 363–364. 1940.
11. RIKER, A. J., and R. S. RIKER. Introduction to research on plant diseases. Planographed by John S. Swift Co., Inc. (St. Louis). 1936.
12. SIDGWICK, N. V. XLIX. Volatility in steam. Benzoic acid and its derivatives. J. Chem. Soc. **117**: 396–406. 1920.
13. VIRTANEN, A. I., and L. PULKKI. The volatility with steam of water-soluble organic substances. J. Amer. Chem. Soc. **50**: 3138–3151. 1928.
14. WALKER, J. C., C. C. LINDEGREN, and F. M. BACHMAN. Further studies on the toxicity of juice extracted from succulent onion scales. Jour. Agr. Res. [U.S.] **30**: 175–187. 1925.
15. ————, and K. P. LINK. Toxicity of phenolic compounds to certain onion bulb parasites. Bot. Gaz. **96**: 468–484. 1935.

FUNGI ASSOCIATED WITH DISEASES OF COTTON SEEDLINGS AND BOLLS, WITH SPECIAL CONSIDERATION OF GLOMERELLA GOSSYPII

R. WEINDLING, P. R. MILLER, AND A. J. ULLSTRUP

(Accepted for publication July 10, 1940)

This paper presents the results of an investigation designed to obtain information regarding the relative distribution and importance of the fungi associated with damping-off of cotton seedlings, and with cotton boll rot.[1]

MATERIALS AND METHODS

Samples of diseased seedlings or bolls were collected at random from individual fields throughout the Cotton Belt, each composite sample being considered representative of the field whence it came.[2] These samples were sent to the laboratory at the South Carolina Agricultural Experiment Station, Clemson, South Carolina, for culturing and identification of the fungi involved.

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[1] This research is based on surveys conducted cooperatively by the Division of Cotton and Other Fiber Crops and Diseases and the Division of Mycology and Disease Survey of the Bureau of Plant Industry. On completion of each separate survey a report has been issued (3, 4, 5, 6).

[2] The writers gratefully acknowledge the cooperation of various individuals who collected and sent in much of the material.

As seedlings were received in the laboratory, 25 were taken from each single sample, thoroughly washed, and the diseased portions trimmed down so as to leave the lesion and a small piece of noninfected hypocotyl. These seedling pieces were surface-sterilized for 5 min. with frequent agitation in a sodium hypochlorite solution containing 1.5–2 per cent available chlorine, and then transferred directly to Petri plates with 10 cc. of hard-water agar. Five pieces were placed in each plate and the plates incubated for 1 week at room temperature (24–27° C.). Microscopic observations on the fungi growing out of the plated tissues were carried out during and at the end of the incubation period. In cases where the identity of a fungus was in doubt, subcultures were made for further study. It is apparent that in surveys of this extent most cultures of the genus *Fusarium* (except *Fusarium moniliforme* Sheld.) could not be identified.

The boll samples were treated similarly. Ten bolls from each sample were used for culturing. A piece of diseased carpel tissue was cut from each boll ($\frac{1}{2} \times 1$ cm.), surface-sterilized, and plated on water agar. In addition, scrapings were made from lesions of bolls, using 3 bolls per sample. If *Glomerella gossypii* (South.) Edg. was not found, more bolls and the bracts were examined. The scrapings were mounted in drops of water on glass slides and observed for spores of the various fungi.

The method of plating on water agar followed by direct microscopic examination deserves repeated emphasis. In the majority of seedling and boll lesions containing *Glomerella gossypii*, this fungus is found to be associated with *Fusarium* or *Alternaria* spp. On ordinary media, such as potato-dextrose agar, these saprophytes overgrow *G. gossypii* in most cases.

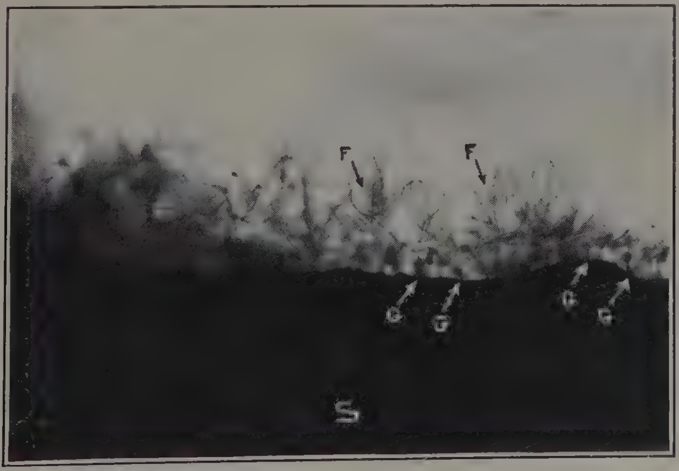

FIG. 1. *Glomerella gossypii* and *Fusarium moniliforme* growing out of a seedling piece. Note tendency of *F. moniliforme* to obscure spore groups of *G. gossypii*. S, seedling piece; G, *Glomerella gossypii*; and F, *Fusarium moniliforme*.

Even on water agar, the profuse development of the fungi is likely to obscure the short conidiophores of *G. gossypii* unless careful focusing of the microscope is employed in observing each plated specimen (Fig. 1).

RESULTS

The most striking finding of this investigation is that, in 1938 and 1939, *Glomerella gossypii* was widely distributed throughout the Southeastern States, as indicated by the percentage of samples of diseased seedlings and bolls in which it occurred (Tables 1, 2, and 3). In addition, this fungus was the predominant organism within most lots of diseased seedlings (Tables 1 and 2). The known virulence of *G. gossypii* as a seedling pathogen justifies emphasis of this fact.

TABLE 1.—*Percentages of Glomerella gossypii isolated from seedlings and bolls during four surveys, in terms of sample lots and individual specimens*

Materials	G. g.[a] on seedlings		G. g. on bolls	
	1938	1939	1938	1939
	Per cent	*Per cent*	*Per cent*	*Per cent*
Sample lots	82.3	89.4	85.8	73.0
Individual specimens	........[b]	56.8	38.1	29.9

[a] Abbreviations for names of fungi used in Tables 1, 2, and 3:
G. g.—*Glomerella gossypii*
Rh. s.—*Rhizoctonia solani*
F. m.—*Fusarium moniliforme*
F. spp.—*Fusarium* species other than *F. moniliforme*
A. spp.—*Alternaria* species.

[b] *Glomerella gossypii* was the predominant organism in 69.7% of the samples. In 1938 data on individual seedlings were not preserved as such but were expressed in terms of predominance of the fungi within sample lots.

Tables 2 and 3 summarize the results from individual States for the 4 groups of organisms most frequently encountered. To facilitate comparison, the data are expressed in terms of percentage occurrence of fungi in sample lots, *i.e.*, the percentage of samples in which the respective fungi occurred one or more times. At the bottom of the tables, the total percentage occurrence in samples is contrasted with that on individual specimens. The occurrence of the following additional fungi was sufficiently common to deserve mention: *Pythium* spp., *Diplodia* spp., *Sclerotium bataticola* Taub., *Sclerotium rolfsii* Sacc., *Aspergillus* spp., and *Chaetomium* spp.

Seedling Study

The similarity of results in the 2 years is evident from table 2. *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.) appeared generally in a low percentage of samples, though sectional and seasonal variations are evident in the figures from individual States, as in Louisiana, with the highest result of 46.2 per cent in 1938. On individual seedlings, *R. solani* was obtained in 1939 only once for each 45 recoveries of *Glomerella gossypii*. *R. solani* is known to be a virulent soil-borne pathogen. Apparently, however, it does

TABLE 2.—*Frequency of occurrence of fungi isolated from diseased cotton seedlings in 1939 and 1938*

1939						1938					
State	No. samples	Percentage of samples yielding				State	No. samples	Percentage of samples yielding			
		G. g.[a]	Rh. s.	F. m.	F. spp.			G. g.[a]	Rh. s.	F. m.	F. spp.
Alabama	49	100.0	16.3	95.9	4.1	Alabama	24	100.0	0.0	87.5	58.3
Arkansas	34	97.1	20.6	97.1	61.8						
Georgia	84	94.0	11.9	95.2	21.4	Georgia	39	100.0	10.3	94.8	43.6
Kentucky	5	80.0	0.0	80.0	20.0						
Louisiana	60	100.0	28.3	91.7	65.0	Louisiana	13	92.3	46.2	46.2	53.8
Mississippi	48	97.9	12.5	79.2	62.5	Mississippi	46	60.9	26.1	45.7	49.3
Missouri	5	100.0	0.0	100.0	40.0						
North Carolina	20	80.0	5.0	65.0	65.0	North Carolina	14	92.9	7.1	92.9	35.7
Oklahoma	17	0.0	0.0	35.3	29.4						
South Carolina	104	86.5	11.5	92.3	38.5	South Carolina	147	75.5	10.2	66.7	38.1
Tennessee	7	100.0	0.0	85.7	85.7	Tennessee	39	89.7	7.7	56.4	71.8
Texas	4	0.0	0.0	0.0	100.0	Texas	4	75.0	25.0	75.0	100.0
Virginia	25	92.0	0.0	96.0	40.0	Virginia	18	100.0	11.1	100.0	55.6
Total	462	89.4	13.2	88.1	41.3	Total	344	82.3	12.8	69.5	46.8
Total no. individual seedlings	11,090										
Percentage seedlings yielding the fungi		56.8	1.4	28.6	6.7						

[a] Abbreviations as in Table 1.

TABLE 3.—*Frequency of occurrence of fungi isolated from diseased cotton bolls in 1939 and 1938*

1939							1938						
State	No. samples	Percentage of samples cultured yielding				Total[b]	State	No. samples	Percentage of samples cultured yielding				Total[b]
		G. g.[a]	A. spp.	F. m.	F. spp.	G. g.			G. g.[a]	A. spp.	F. m.	F. spp.	G. g.
Alabama	15	100.0	86.7	60.0	86.7	100.0							
Arkansas	16	31.3	100.0	87.5	56.2	68.8							
Georgia	34	97.1	88.2	82.4	50.0	97.1	Georgia	7	85.7	100.0	71.4	57.1	85.7
Kentucky	2	100.0	100.0	50.0	100.0	100.0							
Louisiana	14	42.8	85.7	21.4	100.0	92.9							
Mississippi	95	84.2	85.3	78.9	88.4	90.5	Mississippi	36	72.2	47.2	77.8	86.1	91.7
Missouri	5	40.0	100.0	100.0	80.0	60.0							
North Carolina	20	95.0	95.0	85.0	60.0	100.0	North Carolina	27	88.9	88.9	59.3	3.7	96.3
Oklahoma	13	15.4	92.3	69.2	69.2	15.4							
South Carolina	41	78.0	100.0	65.9	53.7	90.3	South Carolina	66	90.9	89.4	81.8	42.4	95.5
Tennessee	6	50.0	100.0	66.7	83.3	50.0							
Texas	17	0.0	100.0	82.4	94.1	11.8							
Virginia	22	90.9	68.2	72.7	31.8	90.0	Virginia	5	100.0	100.0	80.0	60.0	100.0
Total	300	73.0	89.7	73.3	71.3	82.3	Total	141	85.8	79.4	75.9	55.4	94.3
Total no. individual bolls cultured	2,959						Total no. individual bolls cultured	1,762					
Percentage of bolls yielding the fungi		29.9	40.3	20.5	40.3		Percentage of bolls yielding the fungi		38.1	38.9	27.5	21.3	

[a] Abbreviations as in table 1.
[b] Total G. g. includes *G. gossypii* obtained by the scraping method.

not occur in most cotton fields under conditions that make it as destructive as the seed-borne *G. gossypii* (Fig. 2).

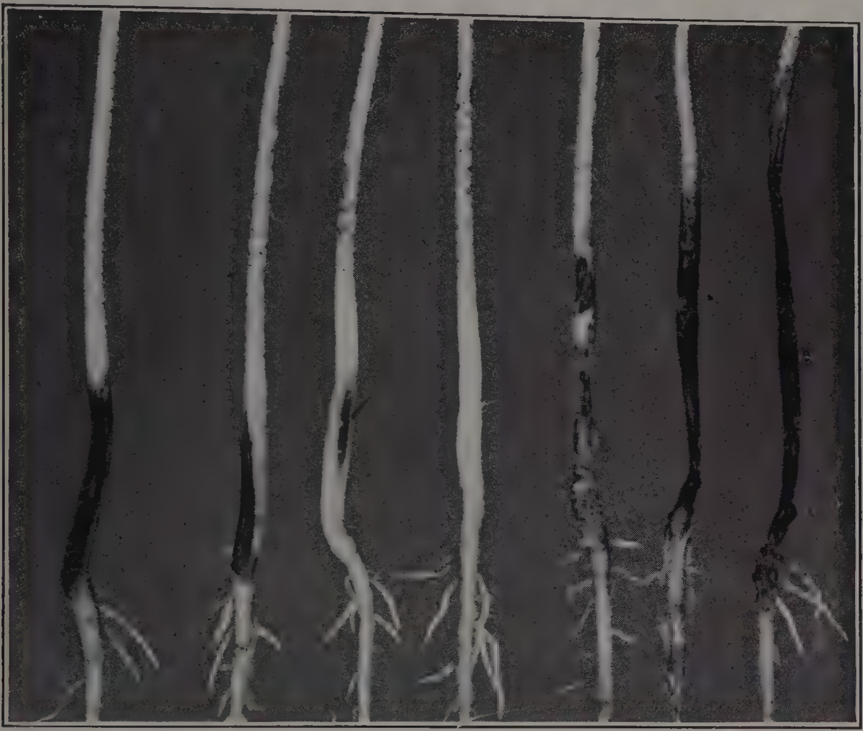

FIG. 2. Damping-off of cotton seedlings caused by *Glomerella gossypii* (left of control plant) and *Rhizoctonia solani* (right). Infection with *G. gossypii* was secured by seed inoculation; that with *R. solani* by infesting the substratum three days after planting the seed. Seedlings of the variety Miller 610 grown for two weeks in flats with steamed sand.

The virulence of *Glomerella gossypii* as a seedling pathogen has been reported by early investigators (1, 2). Recent work with numerous isolates from seedlings (8), and bolls (Weindling, unpubl.) obtained in the present work showed that all were highly pathogenic to cotton seedlings. *Fusarium moniliforme* does not appear to be a primary pathogen of damping-off of cotton seedlings. It has been reported, however, that some of the isolates of this fungus cause root rotting of older seedlings and young plants under certain conditions (9). The fungus is found often on the roots of healthy plants. On diseased seedlings obtained in this survey, it occurred very frequently in association with *G. gossypii*. *Fusarium* often grew out of the seedling piece adjacent to the root. It is difficult at present to evaluate the significance of the high percentage of *F. moniliforme* obtained. The same is true for the various other species of Fusarium isolates, since little is known regarding their pathogenicity to cotton seedlings. Occasionally, *R. solani* was found associated with *G. gossypii*. In such cases it is difficult to say which of the organisms was the primary invader.

Failure to obtain *Glomerella gossypii* from any of the Oklahoma samples is very striking. The organism was found in samples from Texas in 1938 but not in 1939. The 1938 samples, however, were from experimental plots, where seed from other States was used. Moreover, the number of samples from Texas is too small to give these data much significance.

Boll Study

In contrast to the high percentage of seedlings found diseased, lesions on bolls were relatively rare in the field. However, on diseased specimens, *Glomerella gossypii* on bolls was found widely distributed in both years, as indicated by its high percentage occurrence in sample lots (Table 3). The percentage frequency on individual bolls, however, was consistently smaller than on seedlings. In Texas and Oklahoma, the fungus was found in only a few of the eastern counties, 3 in Texas and 1 in Oklahoma. When the data from these 2 States are omitted, 80.4 per cent of samples cultured in 1939 yielded *G. gossypii.* The total percentage (including scrapings) was 90.0.

Alternaria spp. and *Fusarium moniliforme* averaged to occur about as frequently as did *Glomerella gossypii.* There was, however, considerable variation in the occurrence of these fungi from year to year and from State to State. The relatively small number of *Fusarium* spp. noted in 1938 may be attributed to the fact that the 1938 survey was limited to the latter part of the season.

DISCUSSION

The scarcity of Glomerella lesions on cotton bolls, when considered in relation to its abundance as a seedling parasite, requires further consideration. The assumption that the seed is the principal source of the seedling inoculation is supported by the results of seed treatment; but both the small number of boll lesions, as well as the appearance of these lesions, challenge this assumption.

The number of boll specimens observed in advanced stages of rotting was small. Most lesions on the bolls consisted of spots of varying sizes. Spores of *Fusarium* spp. or *Glomerella gossypii* were found rarely on the surfaces of these lesions. Except in such cases, the external appearance of lesions gave no clue as to what fungus might be isolated on plating. Many of these lesions were well-defined, sunken brown spots, often surrounded by irregular water-soaked areas (Fig. 3). Initial lesions, however, consisted either of raised, water-soaked spots of dark-green appearance or of purplish marks and spots. On culturing, these spots, as well as lesions exhibiting more general rot, usually yielded *G. gossypii,* or *Alternaria* spp., or *Fusarium* spp., or any combination of them. Water-soaked spots in the very first stage of development, however, were, in a number of cases, found not to harbor fungi but *Bacterium malvacearum* E.F.S. only. This suggests that *G. gossypii* may often behave like *Alternaria* spp. and *Fusarium* spp., which are considered mainly as secondary invaders entering bolls through lesions

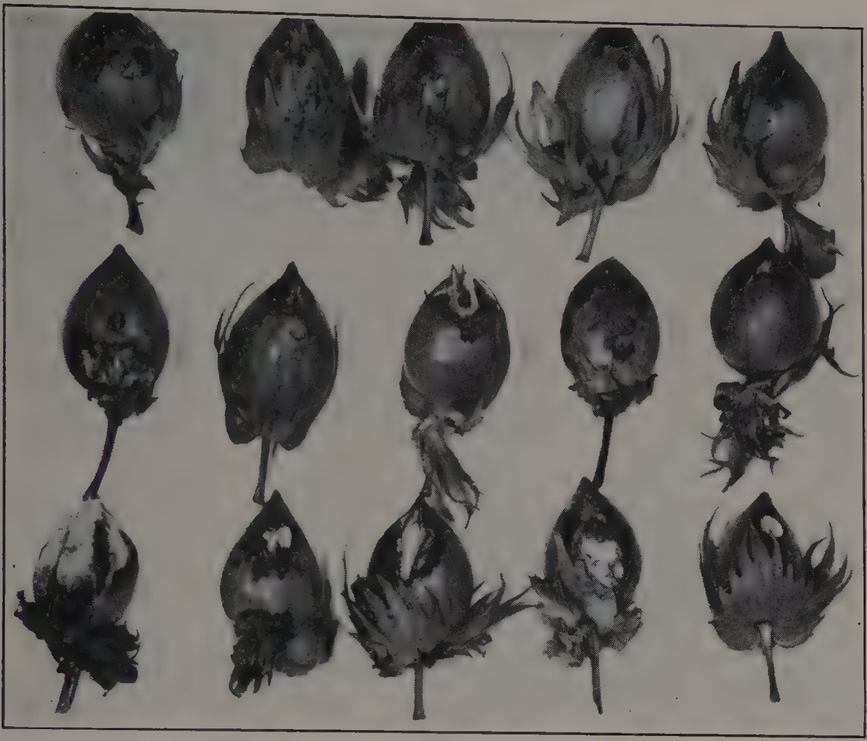

FIG. 3. Bolls collected from a field showing an unusual amount of anthracnose boll rot. Note raised water-soaked spots at the edge of lesions or separate.

caused by other agents. Apparently, boll-weevil punctures are important courts of entry, particularly in the latter part of the season.

Barre (1) and Edgerton (2) published work on anthracnose boll rot about 30 years ago. They discussed sunken, brown spots similar to those described above as intermediate stages of anthracnose at which development may stop under conditions unfavorable for the disease. These authors described the characteristic symptoms of the disease as a severe rot, involving much of the tissue, which becomes covered by the pasty, pink spore masses of the fungus. Conditions at present may be less favorable for the disease than those existing at that time. The large late-maturing cotton of pre-boll weevil days has been replaced by varieties emphasizing early maturity. They lack the abundant foliage that shaded the bolls formerly and provided proper conditions for boll rots. The ability of the anthracnose fungus to invade uninjured tissues of flowers and young bolls was demonstrated by Barre and Edgerton. Probably, the change to the open-type cotton plant decreases the chances for *G. gossypii* as a primary invader.

The survival and spread of *Glomerella gossypii* between seedling- and boll-disease stages also was investigated by Edgerton (2). The fungus was found often in dead portions of stems and leaves of cotton. Observations in 1939 proved all this to be still true. Dead portions of leaves from various fields, when placed in moist chambers, often yielded an abundance of spores of *G. gossypii* within a day or two. Moreover, *G. gossypii* was frequently isolated from lesioned, as well as apparently healthy, stems of cotton plants past the seedling stage. The fungus grew out of surface-disinfected portions of such stems that had been secured from plants in the field and also from plants artificially inoculated at the seedling stage in the greenhouse. It appears, therefore, that *G. gossypii* exists and is disseminated during this period mainly as a saprophyte. This assumption is supported by the fact that the fungus is capable of immediate development and sporulation on dead parts of plants provided sufficient moisture be present.

The scarcity of anthracnose rot on bolls in Oklahoma and Texas is evidently attributable to unfavorable dry conditions, which prevent the fungus from living through this period, producing spore masses, and infecting bolls. The disease was not found in 1939 west of a zone characterized by Thornthwaite as moist subhumid (7) in his maps of climatic provinces based on precipitation effectiveness. Furthermore, percentages of *G. gossypii* on bolls from Arkansas and Louisiana were much lower than on bolls from States farther east, indicating the existence of a transition zone in regard to summer survival.

No difficulty was experienced by the earlier investigators in explaining damping-off due to *Glomerella gossypii,* since *Glomerella* spore loads of 6,000–30,000 per seed were common on their material. Comparable tests (washing of seed followed by centrifuging of the washings) were recently made. In general, much lower spore loads were obtained. Research in progress indicates, however, that small spore loads may bring about a considerable amount of damping-off. Investigations were begun, therefore, to determine whether or not the spores of the fungus are distributed within seed lots and perhaps to other lots by mixing of seed and trash—hulls, unbroken locks, and other debris—during the ginning process. In preliminary studies conidia of *G. gossypii* were obtained in abundance from all trash samples secured from the gins. This apparently accounts for the considerable amount of *Glomerella* on seed obtained from fields in which very little or no anthracnose boll rot was apparent.

SUMMARY

Extensive surveys of diseases of cotton seedlings and bolls have been conducted for 2 years. Random samples were secured from a wide area covering mainly the eastern part of the Cotton Belt. Fungi were isolated from these samples by standardized culture methods.

Glomerella gossypii, which is a virulent seedling pathogen, was the predominant organism in most lots of seedlings from the Southeastern States. The fungus was found widely distributed over this area on bolls as well as on seedlings, generally prevalent on seedlings but relatively rare on bolls. It is emphasized that special methods facilitate identification of the fungus.

Rhizoctonia solani, also known to cause severe damping-off, was isolated

from a relatively small number of seedlings. It was found, however, to be rather widely distributed.

Fusarium spp., including *F. moniliforme* and unidentified species, as well as *Alternaria* spp. were very frequently found on diseased seedlings and bolls.

In this study severe rotting of bolls and the characteristic pink spore masses of *Glomerella gossypii* were rarely observed. Most boll lesions from which *G. gossypii* was isolated were limited spots, indistinguishable from those caused by other microorganisms. It is suggested that this change in type from symptoms described in early literature is due mainly to unfavorable conditions for the disease, brought about by the adoption of early maturing cotton varieties of open type.

The survival and dissemination of *G. gossypii* from the damping-off stage to the boll-rot phase apparently is accomplished through the saprophytic activity of the fungus on dead tissues of stems and leaves of the cotton plant. The scarcity of anthracnose boll rot in Texas and Oklahoma is probably attributable to unfavorably dry conditions preventing the fungus from surviving this period. Transition zones in which anthracnose boll rot decreases from east to west coincide with zones of plant growth, based on climatic features.

Evidence has been obtained that mixing of seed and infested trash during ginning may be responsible for the spread of the fungus on seed. In spite of the relatively small number of diseased bolls, seed may thus be infested sufficiently to cause damping-off of seedlings.

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LITERATURE CITED

1. BARRE, H. W. Cotton anthracnose investigation. S. C. Agr. Exp. Stat. Report **22**: 89–118. 1909.
2. EDGERTON, C. W. The rots of the cotton boll. La. Agr. Exp. Stat. Bull. 13. 1912.
3. MILLER, P. R. A survey of cotton seedling diseases and the fungi associated with them. Pl. Dis. Rptr. **22**: 260–263. 1938.
4. ————. A survey of cotton boll rot diseases and the fungi associated with them. Pl. Dis. Rptr. **23**: 29–32. 1939.
5. ————, and R. WEINDLING. A survey of cotton seedling diseases in 1939 and the fungi associated with them. Pl. Dis. Rptr. **23**: 210–214. 1939.
6. ————. A survey of cotton boll rot diseases in 1939 and the microorganisms associated with them. Pl. Dis. Reptr. **23**: 329–334. 1939.
7. THORNTHWAITE, C. W., B. HOLZMAN, and D. I. BLUMENSTOCK. Climatic research in the Soil Conservation Service. U. S. D. A. Weather Bureau, Mo. Weath. Rev. **66**: 351–368. 1938.
8. ULLSTRUP, A. J. Variability of *Glomerella gossypii*. Phytopath. **28**: 787–798. 1938.
9. WOODROOF, N. CH. A disease of cotton roots produced by *Fusarium moniliforme* Sheld Phytopath. **17**: 227–238. 1927.

MORPHOLOGICAL AND ANATOMICAL FEATURES OF PHYLLODY IN VARIETIES OF TOMATOES AND BEANS[1]

B. F. Dana[2]

(Accepted for publication July 1, 1940)

Investigation of phylloid blossoms produced by big bud in tomato and a similar disease on beans (6) has revealed a close relationship between the morphological change in these blossoms and the vascular anatomy of these species (5). This study was greatly facilitated by published researches on normal floral anatomy by Eames (7), Cooper (4), Guard (8), and Arber (1).

Phyllody (a vegetative change in the flower by which some or all of the parts of the flower become more or less leaflike) appeared in flowers of tomato and bean on branches developing late in the season. In most of the affected plants there was an actual increase and clustering of these branches into witches' brooms. In common bean there was frequently a development of secondary structures (prolification) by the original phylloid flower. The flower in these cases gave rise to secondary or accessory phylloid flowers, inflorescences of phylloid flowers, or normal-appearing shoots.

Extension of the floral axis and modifications of the carpels of tomatoes and beans appeared to follow a pattern determined by the normal floral anatomy of the species. Phyllody and prolification in the bean may furnish information bearing on evolutionary history of the legume carpel about which there has been a difference of opinion (Arber (1), Bancroft (2), Eames (7), Kausik (9), and Saunders (12)).

TRANSFORMATIONS IN PISTILS OF PHYLLOID FLOWERS

Phylloid Carpels in Tomato

In the extreme phylloid or vegetative condition, carpels were represented by individual leaflets. In intermediate forms these leaflets adhered at their margins forming a somewhat lobed, phylloid ovary, pointed at the top (Fig. 1, A and C). Ovaries slightly affected were more rounded in shape, but had a somewhat enlarged and persistent style (Fig. 1, B).

Phylloid Carpels in Beans

In phylloid flowers of common, Lima, and soy bean, transformations in the pistil were all similar. At one extreme the ovary appeared as a wrinkled sac-like pod (Fig. 2). At the other extreme it was changed to a normal-appearing leaf (Fig. 3). Intermediate forms (Fig. 4) occurred in common

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[1] Investigations by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, in cooperation with the Oregon Agricultural Experiment Station and the Division of Irrigation Agriculture of the Bureau of Plant Industry. Investigations conducted at Corvallis, Oregon and at the U. S. Umatilla Field Station, Hermiston, Oregon, which is operated jointly by the cooperating agencies. Published with the approval of the director of the Oregon Agricultural Experiment Station as Technical Paper 336, from the Department of Botany.

[2] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

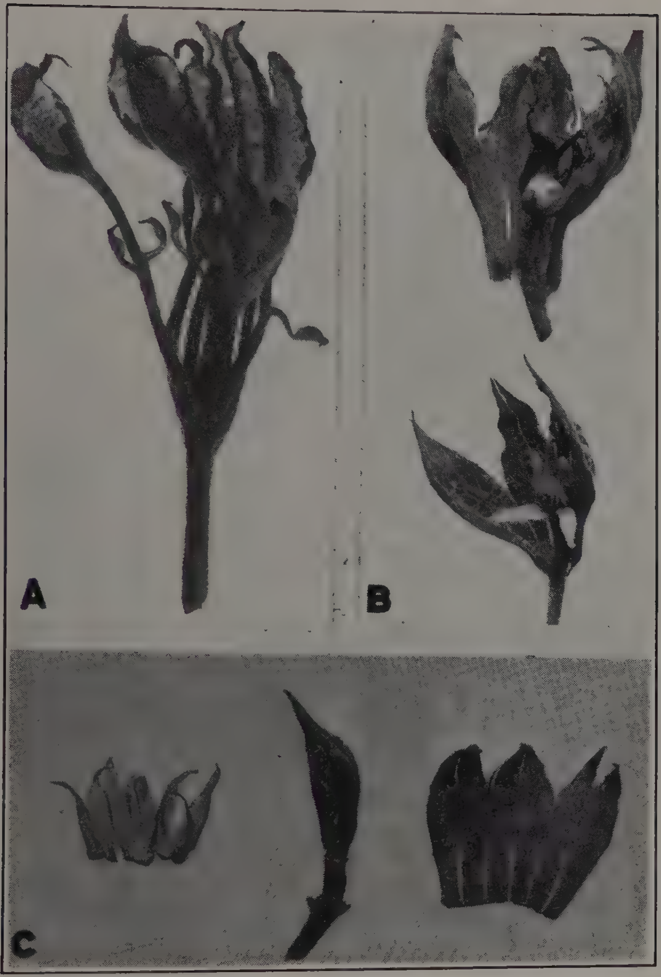

Fig. 1. Phylloid tomato blossoms. A. Calyx and corolla composed of individual phylloid members separated by an extension of the axis. B. Calyx cut and laid back to show axis extension, little changed petals, stamens clinging to ovary, and beaked ovary. C. Phylloid flower dissected to show: left, corolla; center, axis extension between perianth whorls, and beaked ovary; right, united calyx.

and soy bean but, probably because of more limited material, were not observed in Lima bean. In all cases the attachment of the phylloid carpel was lateral on the axis. This is in agreement with the findings of Eames (7) with respect to the normal legume carpel. A range of specimens was available for tracing intermediate forms from the inflated wrinkled pod to the symmetrically-formed and to all appearances normal leaf with petiole.

The Sac-like Ovary. This form was perhaps more common in phylloid flowers than were the expanded leaf forms. It resembled a swollen pod with the pod walls very much wrinkled and folded (Fig. 2, A and B). It had the appearance of a leaf folded upwards on the midrib and joined at the margins in a suture. In many specimens the phylloid ovary had partly

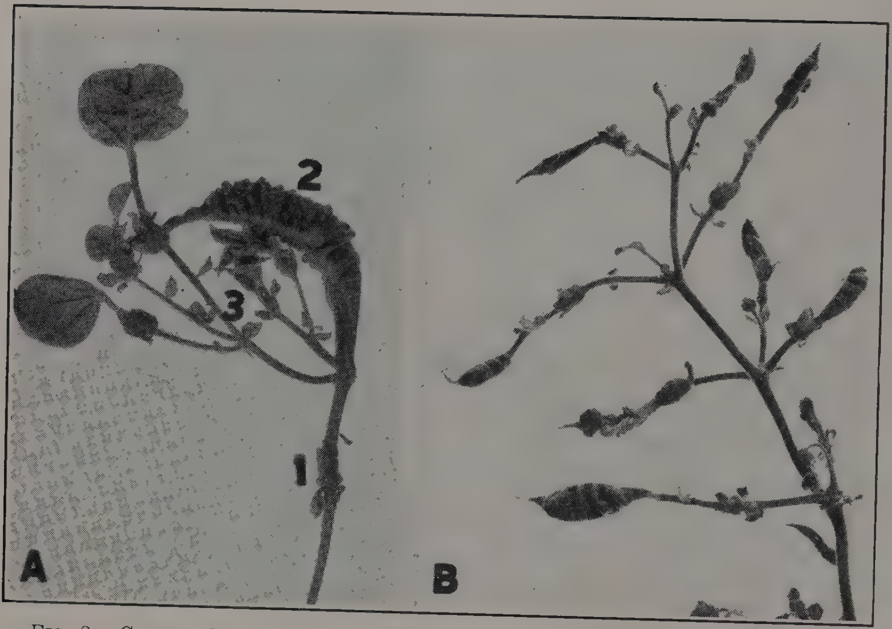

FIG. 2. Common bean. A. (1) Persistent perianth enclosing stamens, and clasping internode—axis extension between stamen whorl and attachment of carpel (2) sac-like wrinkled carpel with attachment clasping node indicating lateral position. (3) secondary inflorescences bearing phylloid flowers. B. Portion of inflorescence showing sac-like phylloid ovaries.

split along the placental suture, usually exposing one or more leaflets attached alternately along the separating margins. With minor variations the sac-like ovary was common in phylloid blossoms of common, Lima, and soy beans. Guard (8) found that in the normal flower of the soy bean the pistil, early in its development, is a leaf-like carpel folded on its midrib with margins scarcely touching and that, before the margins coalesce, protuberances that later give rise to ovules are produced alternately on the inner surface of these carpel margins. This correlates closely with the characters found in legume carpels described above.

Leaf with Marginal Veining. Intermediate between the sac and leaf forms of ovary transformation was a petiolate leaf form with heavy marginal veining. Many of these were cupped and appeared to result from the splitting open of the sac forms. This form could be readily duplicated by carefully dissecting the larger sac forms. Marginal leaflets were always present (Fig. 4). Perhaps because of limited material this form was not observed in Lima bean. It is interesting to note a statement made by Eames (7) that the primitive angiospermous carpel was a palmately veined structure with strongly developed lateral veins and bore marginal ovules. Arber (1) has traced the historical aspects of this concept.

Leaf with Normal Form and Venation. A further transformation in the ovary was represented by a perfectly formed leaf with 3 leaflets (Fig. 3). Intermediate forms exhibited marginal lobes in the main leaflet and, occa-

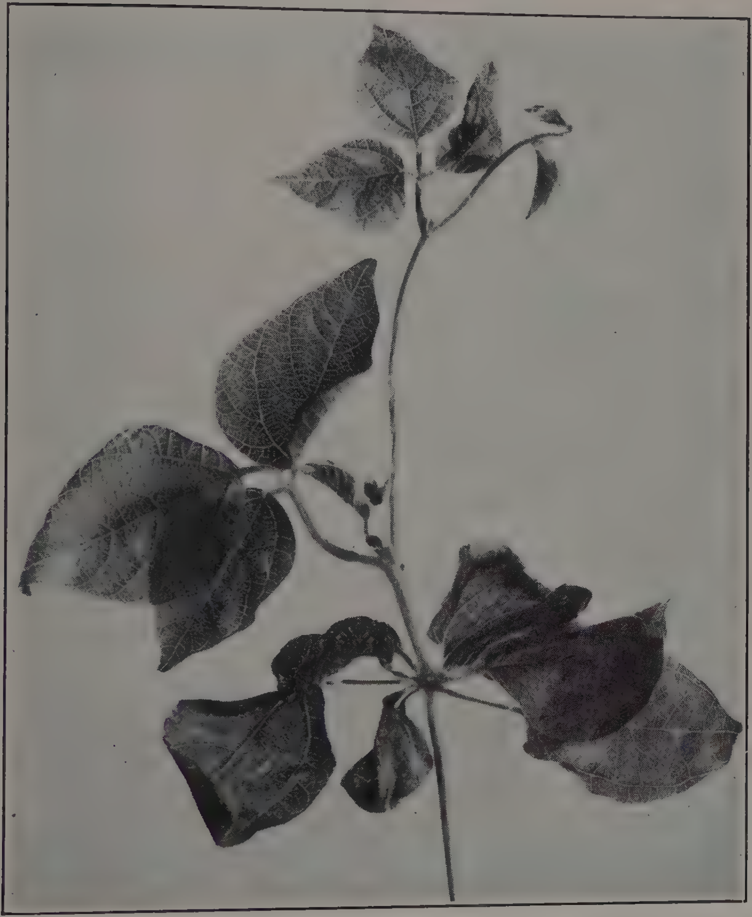

FIG. 3. Phylloid blossom of common bean showing excessive phyllody in perianth, leaf form of phylloid carpel, and accessory shoot extension of the axis.

sionally, partial marginal veining. These intermediate forms indicate that the center leaflet corresponds to the leaf with heavy marginal veining and to the main body of the sac-like forms. The basal leaflets in opposite positions correspond to marginal leaflets reduced in number. The basal leaflets were lacking in many specimens, especially in accessory phylloid flowers (Fig. 2, A).

Ovular Leaflets. These were most symmetrically arranged on the leaf forms with heavy marginal veining (Fig. 4), in alternate fashion along the two heavy marginal veins. Dissections showed that these marginal veins constituted the placental suture of the closed type of transformed ovary. The position of these leaflets in the closed forms corresponds to the position of the ovules in the normal pod. This indicates that these leaflets are analagous in origin to the ovules and may be interpreted as phylloid ovules or "ovular leaflets." In the leaf form of phylloid ovary these leaflets were

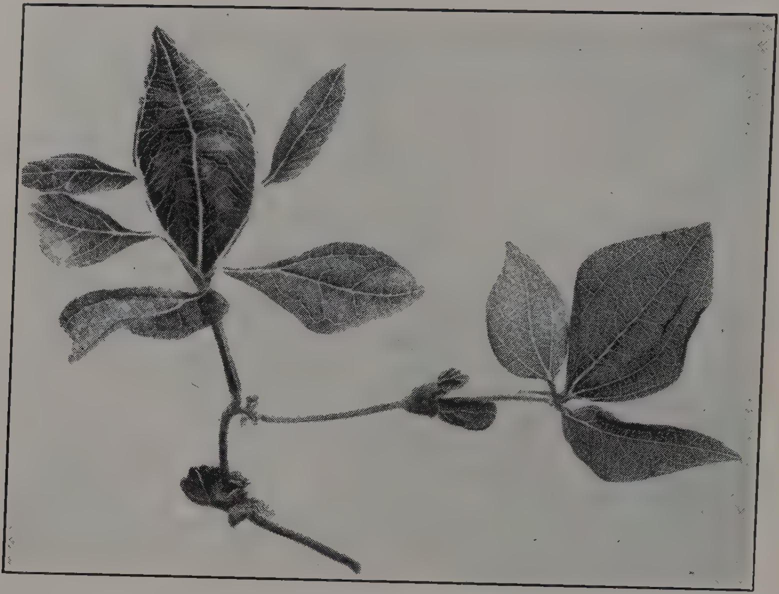

FIG. 4. Phyllody and prolification. Carpel of original flower represented by marginal-veined leaf with ovular leaflets attached in alternate positions. Accessory phylloid flower shows ovary replaced by petiolate three-parted leaf.

reduced to two in number and were situated at the base of the main leaflet. There were intermediate forms with leaflets appearing as lobes of the center leaflet. The heavy marginal veins disappeared simultaneously with the marginal leaflets as these were reduced to two in number, placed at the base of the main leaflet. Bose and Misra (3) have recently described leafy ovules in phylloid flowers of the legume species *Crotalaria juncea.*

EXTENSION OF THE AXIS IN PHYLLOID FLOWERS

Extension between Whorls of the Flowers

In Tomato. In the phylloid tomato blossoms there was a pronounced extension of the axis between the calyx and corolla whorls (Fig. 1, A, B, and C). In external view and cross section the extension was not greatly unlike the pedicel of the individual flower. In some phylloid flowers there were also extensions of the axis between the stamens and the pistil. No cases were observed of extension of the axis beyond the ovary in the form of shoots.

In Beans. No extension of the axis between calyx and corolla whorls was detected in phylloid blossoms of common, Lima, or soy bean. In this respect they differed from the tomato.

An extension of the axis, or formation of an internode, between the stamens and the pistil was evident in phylloid blossoms of several varieties and strains of each of the 3 species of Leguminosae listed above. In common bean (Fig. 2) this extension of the axis or internode was similar in appearance to the pedicel of the phylloid blossom and ranged up to about an inch in length. It was proportionally shorter in the soy bean and Lima bean than in common bean. An internode in the same position was evident in the accessory phylloid flowers borne on the accessory inflorescences.

Accessory Flowers, Inflorescences, and Shoots

Accessory flowers, inflorescences, and shoots were observed in common bean. Single phylloid flowers, or simple inflorescences with phylloid flowers, or both flowers and inflorescences were borne at the node situated at the base of the pistil of the original phylloid flower (Fig. 2, A). These accessory flowers exhibited the same transformations found in the original flowers, although petals in accessory flowers were proportionately larger than in the primary flower. A few instances were observed of accessory flowers that produced other accessory phylloid flowers.

Occasionally, instead of developing as an accessory blossom, the axis was extended into a shoot (Fig. 3) of normal appearance. Excessive vegetative change also was apparent in the perianth of these specimens. Extension of the axis into a shoot is in harmony with the findings of Eames (7) that the legume carpel is lateral, rather than terminal, as is held by Saunders (12).

Anatomical Relationships

Anatomical data for normal flowers are an aid in interpreting axis extensions in phylloid flowers. In a study of the development of the normal tomato flower, Cooper (4) found that stamens are adnate to the throat of the corolla because of early merging of the meristematic zones that give rise to the corolla and stamens. This close association indicates virtual absence of an internode at this point and doubtless explains the absence of axis extension between corolla and stamens in the phylloid tomato flower.

Anatomical studies by Moore (10, 11) show that in normal flowers of the tribe Phaseoleae, vascular elements for perianth, pistil, and disc leave the stele in a combined bundle, which indicates a complete lack of internode, and suggests an explanation for the absence of axis extension in this region of phylloid blossoms of common, Lima, and soy beans. Schaffner (13) calls attention to the fact that in certain species of angiosperms there is an elongated internode between the stamens and the pistil of normal flowers. As examples he cites *Michelia* and species of *Geum*.

MODIFICATIONS IN PERIANTH AND STAMENS OF PHYLLOID FLOWERS

Phylloid Perianth

In Tomato. The calyx in phylloid blossoms of tomato was often greatly expanded, with sepals united. This union of the segments was indicated by clear bands (Fig. 1, C). The greatest enlargement of the calyx appeared in phylloid flowers borne on the older parts of the plant. In these flowers, as well as those on young shoots, the enlarged calyx formed a tube enclosing the other parts of the flower. An apparently rare variation from the enlarged calyx tube was found in one specimen. Here the calyx was represented by narrow and severely streamlined leaves (Fig. 1, A) arranged in a whorl about the axis. Cooper (4), in studying floral development in the tomato, found that primordia for members of the floral whorls are at first separate, which is an aid in the interpretation of the phylloid flowers having individual leaves in the calyx whorl (Fig. 1, A) and those having separate petals (Fig. 1, B).

The corollas of phylloid tomato blossoms usually were not greatly changed in appearance. Segments were united only at the base, if at all (Fig. 1, A, B, and C). Younger blossoms in the inflorescence showed the most enlargement of corolla members. The same specimen that showed separate leaf-like calyx segments also showed phylloid petals.

In Beans. The calyx in phylloid blossoms of common, Lima, and soy beans usually exhibited comparatively little change or enlargement. In the original or primary flower, the calyx was usually cup-shape and partly or completely enveloped the corolla. The only exception observed was in the specimen (Fig. 3) showing extension of the axis into a shoot. In this case, 2 petiolate leaves with single blades represent the calyx.

Primary phylloid flowers of the common bean usually exhibited small, thin petals, scarcely showing above the calyx. In accessory flowers, petals were proportionately more enlarged and leafy. Corolla members of the specimen showing extension of axis into a shoot were greatly enlarged and petiolate.

A more leaf-like condition of petals of phylloid blooms was common in soy bean. There was also a more extensive leafy transformation in the carpels of the soy bean.

Repressed Stamens

Stamens of phylloid tomato blossoms were thin, scale-like, and clinging to the ovary (Fig. 1, B and C). In common bean stamens were persistent and enclosed by the perinath of the phylloid flower. Anthers were normal in shape but appeared empty. There was little indication of expansion in any part of the stamens of phylloid flowers in any of the species of beans studied.

SUMMARY

In phylloid tomato blossoms carpels often were represented by simple leaflets adhering by their margins or fully separated. In bean transformations, the phylloid ovary varied from an inflated, sac-like structure, through a marginal-veined leaf with marginal leaflets representing the ovules, to normal-appearing leaves. There was an extension of the axis between whorls of the phylloid flower where there was not a close relationship (adhesion or union) of vascular traces supplying these adjacent whorls. In common bean there were found secondary or accessory phylloid flowers, inflorescences with phylloid flowers, and shoots produced by axis extension beyond the carpel of the primary flower.

Vegtative modifications of the perianth occurred in both tomato and beans. In extreme cases, members of the perianth whorls were represented by leaf-like structures. Stamens failed to mature normally but persisted, and were separate in the phylloid tomato blossom.

LITERATURE CITED

1. ARBER, AGNES. The interpretation of the flower: a study of some aspects of morphological thought. Biol. Rev. Cambridge Phil. Soc. **12**: 157–184. 1937.
2. BANCROFT, HELEN. A review of researches concerning floral morphology. The Bot. Rev. **1**: 77–99. 1935.
3. BOSE, R. D., and S. D. MISRA. Studies in Indian fibre plants. No. 6. Phyllody and some other abnormalities in the flower of sunn-hemp. Indian Jour. Agr. Sci. **8**: 417–423. 1938.
4. COOPER, DELMER C. Anatomy and development of the tomato flower. Bot. Gaz. **83**: 399–411. 1927.
5. DANA, B. F. Morphological and anatomical features of phyllody in varieties of tomatoes and beans. (Abstract) Phytopath. **29**: 823. 1939.
6. ————. Occurrence of big bud of tomato in the Pacific Northwest. Phytopath. **30**: 866–869. 1940.
7. EAMES, ARTHUR J. The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. Amer. Jour. Bot. **18**: 147–188. 1931.
8. GUARD, ARTHUR T. Development of floral organs of the soy bean. Bot. Gaz. **91**: 97–102. 1931.
9. KAUSIK, S. B. Morphology of abnormal flowers in some angiosperms. New Phytol. **37**: 396–408. 1938.
10. MOORE, JOHN ADAM. The vascular anatomy of the flower in the papilionaceous Leguminosae. I. Amer. Jour. Bot. **23**: 279–290. 1936.
11. ————. The vascular anatomy of the flower in the papilionaceous Leguminosae. II. Amer. Jour. Bot. **23**: 349–355. 1936.
12. SAUNDERS, EDITH R. Illustrations of carpel polymorphism. IV. New Phytol. **23**: 225–258. 1929.
13. SCHAFFNER, JOHN H. The fundamental nature of the flower. Bull. Torr. Bot. Club **64**: 569–582. 1937.

EFFECT OF X-RADIATION ON THE GERMINATION OF CHLAMYDOSPORES OF USTILAGO HORDEI

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Previously, X-radiation of smut fungi has been studied primarily as a means of controlling the organisms. Pichler and Wöber (9) have reported control of *Tilletia tritici* (Bjerk.) Wint. by X-radiation, but data presented by Tascher (10) indicate that, in general, differentials in the killing points of the hosts and smut fungi usually are not large enough to control the parasite without serious injury to the host. A review of the literature, together with original data on X-ray treatments for controlling other fungi, has been presented by Tascher (10). Studies on the effect of X-radiation on sectoring in fungus cultures also have been reported. Leonian (5) obtained negative results with a large number of the Fusaria as did Johnson (7) with cultures of *Sclerotium bataticola* Taub., *Collybia dryophila* Fr., and *Fusarium batatatis* Wr. Dickson (2), however, induced sectoring in cultures of *Mucor genevensis* Lend., *Phycomyces blakesleeanus* Burgef., and *Chaetomium cochliodes* Pall. by X-radiation. X-rays, up to 6-hour exposures, had little effect on the germinating capacity of the latter organism. Irradiation, however, affected the mycelial growth from these spores, development becoming less with increased exposure up to a maximum of 4 hours. Uber and Goddard

(11) studied the effect of x-radiation on *Neurospora tetrasperma* S. and D. and found that the normal growth processes were inhibited at much lower dosages than those affecting germination. In the present paper results are reported on the effect of X-rays on chlamydospore germination, promycelial and sporidial development, and sectoring in *Ustilago hordei* (Pers.) K. and S.

MATERIAL AND METHODS

An air-cooled, thin-wall, Coolidge X-ray tube, with tungsten anode operating under a constant potential of 45,000 volts, gave a continuous band of radiation used with maxmium intensity at approximately 0.50 Å and a short wave length limit of 0.27 Å. The anode distance was 13 cm., the tube current 7 milliamperes with exposures up to 25 hours. Dosage values were measured with a standard open-air ionization chamber (6). The symbol "kr." is used throughout this paper for the abbreviation of a recently defined[1] dosage unit, the kilo-roentgen, equal to 1000 roentgen units. Errors in dosage measurement were less than 5 per cent. The chlamydospores of *Ustilago hordei* subjected to X-ray treatment were spread in a thin layer on the bottom of paper cups approximately 1 cm. square, resting on a cheesecloth diaphragm, to reduce the amount of X-ray back-scattering.

For germination tests and studies on promycelial and sporidial development, irradiated and nonirradiated chlamydospores were spread on 2 per cent potato-dextrose agar in Petri dishes and incubated at 18° C. Germination counts were made on 400 chlamydospores for each dosage value and any protrusion of the promycelium was considered an index of germination. Heidenhain's iron-alum haematoxylin was used as a nuclear stain. The procedure was the same as that outlined by Holton (4) for staining nuclei of the oat-smut fungi except that diaphanol was substituted for hydrogen peroxide as a bleaching agent.

RESULTS AND DISCUSSION

Chlamydospore Germination

The effect of various dosages of X-rays on germination of chlamydospores is shown graphically in figure 1. The percentage of germination of nonirradiated spores was exceptionally high and was not reduced materially for dosages up to 100 kr. Above this point the time required for germination and the number of dead spores increased gradually from dosages of 150 to 300 kr., measured at 50-kr. intervals. There was a marked decrease in the percentage survival at 350 as compared with 300 kr. Chlamydospores exposed to 300 kr. germinated 72 per cent during 17 hours and 80 per cent during 24 hours' incubation, while the germination of those exposed to 350 kr. was only 2 and 37 per cent, respectively. Ten per cent germination occurred when the spores were exposed to 500 kr., but above this point only a trace of germination was observed. Counts made after 48 hours' incubation were not significantly different from those after 24 hours.

[1] Kempton, J. H., and L. R. Maxwell. Effect of temperature during irradiation on the x-ray sensitivity of maize seed. Manuscript submitted to Jour. Agr. Res.

There is an indication from the following data that some physiological condition of the spore determines the degree to which certain X-ray dosages affect spore germination. Chlamydospores for the above experiment were obtained from Trebi barley. A portion of the nonirradiated spore mass was used to reinoculate this variety and the resulting chlamydospores were irradiated. Results obtained with this second lot of spores are indicated by the large dots in figure 1. It will be noted that percentage germination of the nonirradiated spores was slightly higher than in the previous experiment, but somewhat lower when dosages up to 100 kr. were used. Greater differences appeared when the exposure was 500 kr. In this test 50.6 per cent of the spores germinated during 24 hours of incubation as compared with 10 per cent for the original material.

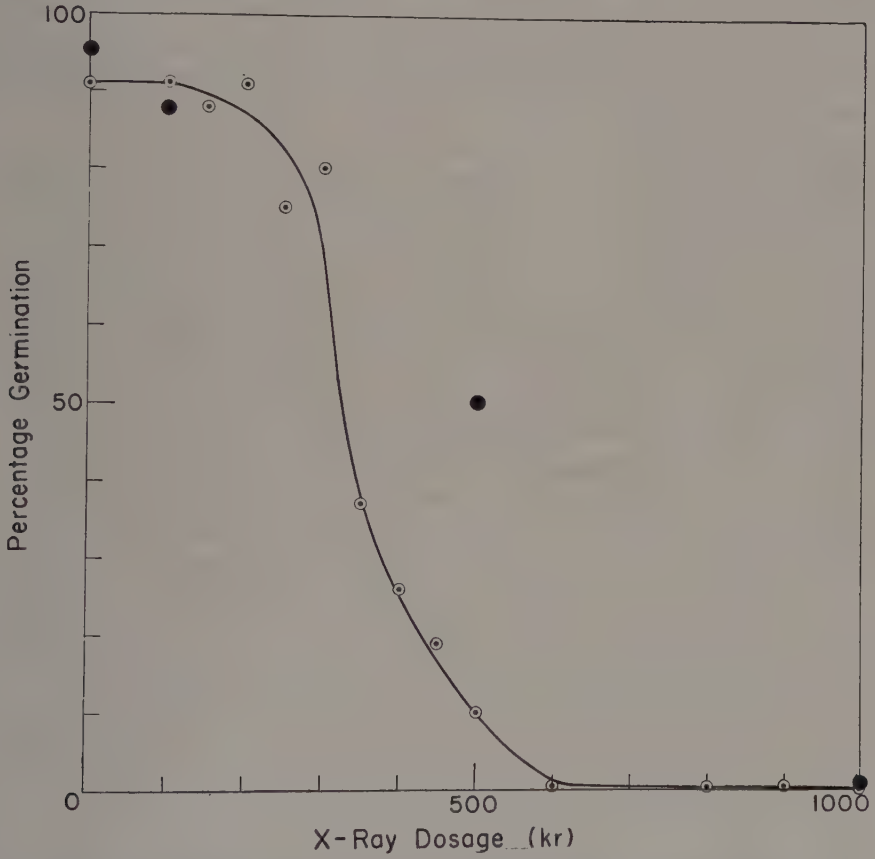

FIG. 1. Germination of nonirradiated chlamydospores of *Ustilago hordei* and of those x-rayed at dosages from 1 to 1000 kr. Large noncircled dots represent a group of spores that presumably differed in degree of physiological maturity.

Promycelial and Sporidial Development

A study was made of the promycelial and sporidial development from chlamydospores irradiated at each of the dosages indicated in figure 1. Uber and Goddard (11) observed that with increasing x-ray dosage up to 100 kr. progressively there was a decrease in the length of the germ tube of *Neurospora tetrasperma,* agreeing with observations of Dickson (2) on *Chaetomium cochliodes* and Luyet (8) on *Rhizopus nigricans* Ehr. With *Ustilago hordei* no effect on the promycelium was noted when the chlamydospores were irradiated at 1 or 10 kr. but at 30 kr. there was a tendency toward elongation of the promycelium, which became progressively more marked with dosages of 60, 100, and 150 kr. Promycelia from chlamydospores given 150 kr. were from 1.5 to 3 times as long as those from nonirradiated spores. At 200 kr. there was no consistency in their development, the lengths varying from a slight protrusion of the promycelium to twice normal length. With still higher dosages the average length decreased until at 500 kr. none greater than normal length was observed. Luyet (8) noted the presence of giant cells resulting from X-radiation of spores of *R. nigricans.* No such abnormality was observed among the irradiated chlamydospores of *U. hordei* nor in the limited number of haploid lines that were subjected to low dosages. Camera-lucida drawings of germinated nonirradiated and X-rayed chlamydospores are shown in figure 2.

Observations of the effect of X-rays on sporidial formation revealed a slightly delayed development at 30 and 60 kr., but a normal number of sporidia at all dosages up to 60 kr. At 100 kr., sporidia developed on only about 10 per cent of the promycelia with not more than 2 on any promycelium. An occasional sporidium developed from chlamydospores receiving dosages up to 600 kr. and these increased in number by budding in the normal manner. Fused sporidia with infection hyphae were observed in certain cultures of paired monosporidial lines isolated from the 200 kr. material.

In *Neurospora tetrasperma* the normal growth process is inhibited by X-radiation at much lower dosages than those required to destroy germination (11). This appears to occur also in *Ustilago hordei,* for, although as high as 80 per cent germination of chlamydospores was obtained in material irradiated at 300 kr., the normal number of primary sporidia was not found at dosages above 60 kr. Promycelia that did not form sporidia ceased to elongate after a 24-hour incubation period and became vacuolated, indicating they were dead. Inhibition of the growth process after germination resulting from X-radiation has been observed in corn seedlings by Collins and Maxwell (1), and described by them as "delayed killing." This term might be used to describe the same process in promycelia of *U. hordei.*

In *Ustilago hordei,* abnormal elongation of the promycelium and reduction in number of sporidia accompanied an abnormal nuclear division and nuclear migration in the promycelium. Nonirradiated material of *U. hordei* was stained at different stages of germination and the process of nuclear division and migration was observed to be similar to that described by Hanna (3) for *U. zeae* (Beck.) Ung. Thus, after meiosis and a division of the two daughter nuclei, each segment of the promycelium contains a single haploid nucleus and, as indicated in figure 2, A and B, each may produce a primary sporidium. In contrast, the chlamydospores irradiated at 200 kr., with few exceptions, developed elongated, nonseptate promycelia. No nuclei were present in a few of these promycelia, several had 1 and most of them had 2 instead of the 4 nuclei found in the nonirradiated material. Nearly all of these promycelia produced no primary sporidia. An occasional elongated promycelium with a single sporidium was observed and one had two primary sporidia, as shown in figure 2, E.

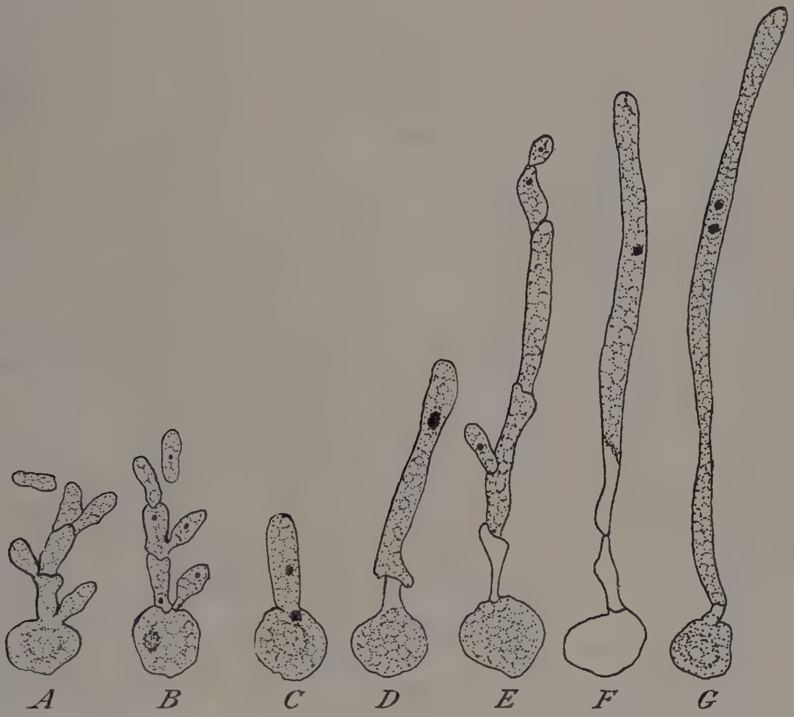

FIG. 2. Type of germination of chlamydospores of *Ustilago hordei* incubated 24 hours. A and B. Nonirradiated; C to G, irradiated 200 kr.

Mutation

In the experiments on sector formation a single primary sporidium was isolated from the promycelium of each of 50 chlamydospores that were (1) nonirradiated; (2) irradiated 50 kr., and (3) irradiated 100 kr. Each single sporidial line was increased under comparable conditions, then subcultured and grown for 35 days in duplicate 250 cc. flasks on 35 cc. of 2 per cent Difco potato-dextrose agar. During this time the laboratory temperature varied between 20° and 26° C. The number of sectors per flask of monosporidial lines originating from the nonirradiated chlamydospores varied from none to 4 with totals of 51 and 49 sectors in duplicate series of 50 lines. In the irradiated material the number of sectors per flask varied from none to 3, and the total sectors for the duplicate series originating from chlamydospores receiving 50 kr. were 39 and 62, and for those receiving 100 kr., 48 and 60 sectors. It is thus evident that under the conditions of these experiments X-radiation failed either to induce or inhibit sectoring. Furthermore, variation in the general color and growth types in colonies of monosporidial lines from irradiated chlamydospores was no more marked than in the lines from nonirradiated chlamydospores.

SUMMARY

The effect of X-radiation of chlamydospores of *Ustilago hordei* on germination, sporidial development, and frequency of sectoring was measured. Percentage of germination was not materially affected by dosages up to 100 kr. At higher dosages the number of nonviable spores and the time required for germination of the viable ones increased irregularly with increasing dosage up to 1000 kr., at which all of the spores were killed. There is some evidence that the degree of X-ray sensitivity depends on some unknown physiological condition of the chlamydospores.

Irradiation of the chlamydospores at certain dosages resulted in an increased elongation of the promycelia. It was noticeable at 30 kr. and progressively more marked at 60, 100, and 150 kr. At the latter dosage promycelial lengths were approximately 3 times normal. Septation was rare. At 200 kr. and above, promycelial lengths varied from approximately .5 to 3 times normal length. Promycelia from chlamydospores irradiated up to 60 kr. developed a normal number of primary sporidia. At dosages of 100 kr. sporidia developed on only approximately 10 per cent of the promycelia and at dosages of 150 kr. and above, only an occasional sporidium was observed.

The normal growth processes in *Ustilago hordei* are inhibited by X-radiation of lower dosages than is required to destroy the mechanism responsible for germination. Although as much as 80 per cent germination was obtained with chlamydospores irradiated at 300 kr., development ceased after elongation of the promycelium. It is suggested that this effect be called "delayed killing."

X-irradiation of chlamydospores at 50 and 100 kr. did not affect the rate of mutation in cultures of monosporidial lines of *Ustilago hordei*.

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LITERATURE CITED

1. COLLINS, G. N., and L. R. MAXWELL. Delayed killing of maize seedlings with x-rays. Science 83: 375–376. 1936.
2. DICKSON, H. The effects of x-rays, ultraviolet light, and heat in producing saltants in *Chaetomium cochliodes* and other fungi. Ann. Bot. 46: 389–405. 1932.
3. HANNA, W. F. Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. 19: 415–442. 1929.
4. HOLTON, C. S. Studies in the genetics and cytology of *Ustilago avenae* and *Ustilago levis*. Minn. Agr. Exp. Stat. Tech. Bull. 87. 1932.
5. LEONIAN, L. H. Studies on the variability and dissociation in the genus *Fusarium*. Phytopath. 19: 753–865. 1929.
6. MAXWELL, L. R., and S. B. HENDRICKS. X-rays in agriculture. Jour. Appl. Phys. 9: 237–243. 1938.
7. JOHNSON, F. H. Effects of electromagnetic waves on fungi. Phytopath. 22: 277–300. 1932.

8. LUYET, B. J. The effects of ultra-violet, x-, and cathode rays on the spores of Mucoraceae. Radiology 18: 1019–1022. 1932.
9. PICHLER, F., and A. WÖBER. Bestrahlungsversuche mit ultraviolettum Licht, Röntgenstrahlen und Radium zur Bekämpfung von Pflanzenkrankheiten. Centbl. Bakt. 57: 319–327. 1922.
10. TASCHER, W. R. Experiments on the control of seed-borne diseases by x-rays. Jour. Agr. Res. [U.S.] 46: 909–915. 1933.
11. UBER, F. M., and D. R. GODDARD. Influence of death criteria on the x-ray survival curves of the fungus, *Neurospora*. Jour. Gen. Phys. 17: 577–590. 1934.

THE SHAPE AND DEVELOPMENT OF POTATO TUBERS, AND THEIR SIGNIFICANCE IN THE DIAGNOSIS OF SPINDLE TUBER

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In American descriptions of potato spindle tuber, a characteristic but not invariable symptom in many varieties is said to be elongation of the tubers (5, 6, 3). A condition in Factor (Up-to-Date) potatoes in New South Wales, which has been diagnosed as spindle tuber (4), causes significant reduction of cross section in tubers of commercial size, but not in small ones. Measurements on diseased and normal material have led to interesting generalizations on the shape and development of potato tubers, and the effect on them of this disease.

METHODS

The measurements were mostly obtained from tracings of the tubers made by pricking an outline on paper with a needle carried in a long thin mount. Direct measurements with calipers were sometimes used, and the results were in close agreement with those obtained from tracings. The measurements were of length and 6 cross-sectional diameters, 3 in each of 2 planes at right angles, the planes corresponding to the wider and narrower diameters of the tubers. The 2 planes represent what Schultz and Folsom (5) designate as width and depth. The measurements were made at $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ the distance from the stem to the bud end. The 3 pairs of measurements allowed detailed analyses of tuber shape (not presented here), and gave mean figures of cross-sectional diameter. The mean figures were less subject to variations due to minor irregularities of contour than were single pairs of measurements. From the cross-sectional diameters was obtained a figure for relative cross-sectional area, by converting the 6 values to logarithms, adding, and dividing by 3. This gave a logarithmic mean, easily obtained, convenient for calculation, and in practice very little different from an arithmetic mean. The relative cross-sectional area was so closely correlated with the real area that it was used freely in its place. Relative cross-sectional areas were compared with lengths by plotting them on a double logarithmic scale, and calculating regressions of relative cross-section on length, using the log values.

RESULTS

Figure 1 includes a summary of the results obtained with tubers of the variety Factor (Up-to-Date) free from spindle-tuber, and infected with spindle tuber. The summaries take the form of curves that were fitted to the measurements of 161 tubers of noninfected plants, and 81 tubers of infected plants. The interpretation of the curves depends on the fact that measurements of tubers swelling equally in all dimensions, if plotted in this way, give a straight line of slope 2; if the tubers increase more rapidly in length than in other dimensions, the slope is less than 2; if increase in cross-sectional area is proportional to increase in length, the slope is 1.

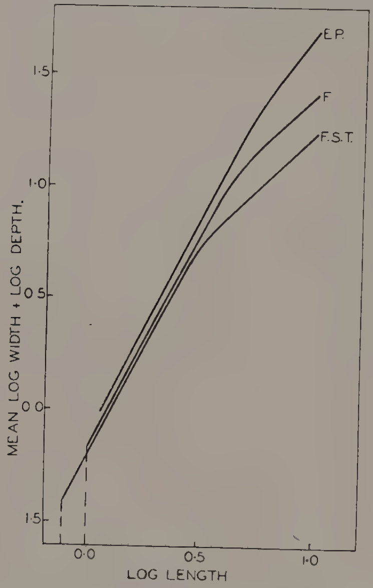

FIG. 1. Curves to illustrate the development of normal tubers of the variety Factor (F), of tubers from Factor plants affected with spindle tuber (F.S.T.), and tubers from normal plants of the variety Epicure (EP.). The curves F and F.S.T. should more nearly coincide in the range below log. 1 = 0.5 than is shown; they have been slightly separated to make the diagram clearer.

The curves in figure 1 are composite. The curve for normal Factors is derived from 2 straight lines, one fitted to data for tubers between 1 cm. and 5 cm. in length, and the other to tubers above 5 cm. The difference between the slope of the two lines is highly significant. The dividing point between larger and smaller tubers is taken where it is because the inclusion of measurements from tubers longer than 5 cm. reduces the slope of the regression line below that obtained from measurements of smaller tubers alone.

The curve for normal Factors indicates three stages in the growth of the tubers:

1. The stolon tips swell to a rudimentary tuber (1) about 1 cm. in length, and slightly less than .9 cm. at its widest point radially. Normally these rudiments do not vary greatly from a size characteristic for healthy plants of the variety. The first stage is suggested

diagrammatically in figure 1 by a vertical dotted line. Whether, as a literal interpretation of this line would suggest, the stolon expands radially without any increase in length over the expanding area, has not yet been determined.

2. In the second stage the tuber increases in length to about 5 cm. without changing significantly its relative dimensions. The slope of the line between log. $l = 0.0$ and log. $l = 0.7$ is 1.895, which is not significantly different from a slope of 2, although the t-test shows the differences to be not far from the 1-in-20 level of significance. This stage is partly accounted for by swelling of the cells already formed in the tuber when it is about 1 cm. long, but there is also probably an increase in cell number. From the experiments of Fellows (2), which have been repeated by the writers, it seems that, even in this stage, the outermost layers of cells towards the stem end do not accommodate themselves to the increase in size, by expansion or multiplication, so much as do those towards the apex of the tuber.
3. In normal tubers of the variety Factor there is a rapid transition from the second to the third stage. In the latter, the length remains approximately proportional to the cross-sectional area (b, the regression coefficient, $= .971$), *i.e.*, apical growth becomes relatively more important.

Evidence has been obtained from periodical measurements on growing tubers attached to the plant that the regression line between the points log. $l = 0.0$ and log. $l = 0.7$ represents the type of expansion that actually occurs in individual tubers, but similar measurements have not been made on tubers expanding to greater size. Until they have been made, the straight line regression above log. $l = 0.7$ cannot be claimed to represent the continuous growth of individual tubers, but is merely a mean relation between cross-sectional area and length of mature tubers above 5 cm. in length.

The curve for the tubers from Factor plants diagnosed as infected with spindle tuber illustrates the same 3 stages, with the following differences:

a. The rudimentary tubers formed from the stolon tips are of the same shape as the rudiments of normal plants, but appear to be smaller.
b. They grow uniformly in all dimensions from about .75 cm. (this figure is subject to correction, as it depends on only a few measurements) to 2.5 cm. in length; *i.e.* expansion in the second stage is relatively less than expansion of tubers from normal plants.
c. There is a transitional zone between the second and third stages, and, thereafter, growth appears to follow a course similar to that of normal tubers; but, as predominantly apical growth begins in smaller diseased tubers, these tubers are narrower in cross-section than healthy tubers of the same length.

The relation between cross-sectional area and length, for tubers of commercial size, has been found to show little if any systematic variation under contrasting conditions of growth, and has been used on several occasions to distinguish between yields of diseased and normal plants. Extreme conditions of competition and freedom from competition may possibly affect the relation.

Schultz and Folsom (5) found that mosaic in Green Mountains does not affect tuber shape. Measurements made on President tubers grown at Canberra, containing virus X and free from it, indicate no differences in shape due to infection. Similarly, tubers of Factor and of a variety incorrectly named Delaware, which is grown mainly in Western Australia, are unaffected in shape by leaf roll. Factor tubers normally carry virus X and virus B, and Delaware virus X, but not virus B. It appears that of the more widespread viruses of the potato in Australia, only spindle tuber is likely to cause changes in tuber shape.

COMPARISON WITH MEASUREMENTS ON GREEN MOUNTAIN AND BLISS TRIUMPH TUBERS

Previous data on the shape of normal tubers and those affected with spindle tuber have mainly been put in the form of a simple ratio between length and width (5, 6, 3). It is interesting to find that measurements of Green Mountain tubers of marketable size, both healthy and diseased with spindle tuber, which are tabulated by Schultz and Folsom in the form of a frequency table (5, p. 57), give results very similar to those derived from our measurements, and so also do measurements made on Bliss Triumph by Werner (6, Fig. 2). By reducing the classes of Schultz and Folsom's tables to fit into equal increments on a logarithmic scale, the following regression coefficients for log. width on log. length are obtained:

Diseased material	.447
Healthy material	.488

Neither regression coefficient is significantly different from a value of 0.5. Werner's data show apparently aberrant shapes among the healthy Bliss Triumph below 4.5 cm. in length, but longer tubers, both healthy and diseased, give regression coefficients that do not differ significantly from 0.5. When our data for Factor tubers in the third stage of development are reduced to two measurements, length and width at the center, the regression coefficient is also not significantly different from 0.5. If the tuber dimensions are put in the form $w/\sqrt{l}$ or w^2/l, a constant is obtained that is independent of size of tuber within certain limits. Thus, direct comparison can be made between healthy and diseased tubers of these 3 varieties by means of a single constant, which may be given either as k of the following equation:

$$w - \tfrac{1}{2} \log. l = k \qquad 1$$

where w is the width of the tuber, and l the length, or as the ratio $w/\sqrt{l}$. The values obtained for Green Mountain, Bliss Triumph, and Factor are as follows:

	Green Mountain		Bliss Triumph		Factor (Up-to-Date)	
	Normal	S. T.	Normal	S. T.	Normal	S. T.
k	.39	.27	.37	.31	.30	.20
$w/\sqrt{l}$	2.45	1.86	2.34	2.04	2.00	1.58

The reduction in cross-section of diseased tubers below that of healthy tubers of equal size appears greater for the varieties Green Mountain and Factor than for Bliss Triumph. Green Mountain and Bliss Triumph tubers are normally shorter in relation to width than Factor, and diseased tubers of these two varieties are not very different in their dimensions from normal Factor, except that they are rounder in cross-section. The rounding of cross-section, so typical of Green Mountain tubers affected with spindle tuber, is found in Factor, affected with spindle tuber, mainly under conditions likely to cause second growth, but comparatively little rounding is found when growing conditions are exceptionally favorable. The same appears to be true for Bliss Triumph (6).

TUBERS OF THE VARIETY EPICURE

If the growth of tubers of other varieties follows a course similar to those of Green Mountain and Factor throughout the third stage of development, k of equation 1, or K of equation 2 below will provide a simple index of shape for tubers of commercial size, by which varieties may be characterized:

$$\text{Log. } a - \text{log. } l = K, \qquad 2$$

where a is the relative cross-sectional area.

The curve for the round English variety, Epicure, in figure 1 shows that equation 2 is not universally applicable. The tuber rudiment is relatively greater in cross-section than that of Factor, it expands equally in all dimensions to a greater size, and, in the third stage, apical growth is less in comparison with growth in cross-section—the slope of the regression line above log. $l = 0.8$ is 1.28, a value significantly greater than l. The equation becomes:

$$\text{Log. } a - 1.28 \text{ log. } l = K,$$

and a single constant fails to describe the relation between length and cross section of large tubers. However, tubers of the varieties Factor and Epicure go through three similar stages of development, and may be characterized by measurements of their relative dimensions. Such measurement might prove a useful method in the study of the physiology of growth of potato tubers and in the genetics of tuber shape, as well as in the original object of this investigation, the diagnosis of a virus disease, and its closer identification with American spindle tuber.

SUMMARY

Measurements of potato tubers of the variety Factor (Up-to-Date) indicate 3 stages in their development from stolon to the mature tuber.

A disease in Australian potatoes, diagnosed as spindle tuber, causes a decrease of cross-sectional area relative to length in tubers of marketable size. The shape of small tubers is unaffected.

Several other viroses and a variety of environmental conditions fail to alter tuber shape.

An analysis of measurements made on Green Mountain tubers by Schultz and Folsom and on Bliss Triumph tubers by Werner gives results very similar to those obtained from Australian material.

Varieties may be characterized by the dimensions of their tubers if the stages of tuber development are taken into account.

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LITERATURE CITED

(1) ARTSCHWAGER, ERNST. Studies on the potato tuber. Jour. Agr. Res. [U.S.] **27**: 809–835. 1924.

(2) FELLOWS, H. Relation of growth in the potato tuber to the potato scab disease. Jour. Agr. Res. [U.S.] **32**: 757–781. 1926.

(3) GOSS, R. W. The symptoms of spindle tuber and unmottled curly dwarf of the potato. Nebr. Agr. Expt. Stat. Res. Bull. 47. 1930.

(4) NOBLE, R. J., et al. The occurrence of plant diseases in New South Wales. Dept. Agr. N.S.W. Sci. Bull. **57**: 1–42. 1937.

(5) SCHULTZ, E. S., and DONALD FOLSOM. Transmission, variation, and control of certain degeneration diseases of Irish potatoes. Jour. Agr. Res. [U.S.] **25**: 43–117. 1923.

(6) WERNER, H. O. The spindle-tuber disease as a factor in seed potato production. Nebr. Agr. Expt. Stat. Res. Bull. 32. 1926.

EYE SPOT OF LEMON GRASS

B. A. BOURNE

(Accepted for publication July 19, 1940)

During the past 6 years a leaf spot of lemon grass, *Cymbopogon citratus* DC., was observed to be common throughout the year in the Florida Everglades. The spots resembled the common eye spot of sugar cane, as described by Faris,[1] and by the writer for the early stages of ring spot of sugar cane,[2] although the India-red colors, common in connection with the cane disease, were lacking.

Until 1938, when Voorhees[3] described an eye spot of napier grass in Florida, and concluded from his experiments that the causal organism of this disease was identical with *Helminthosporium ocellum* Faris, the sugarcane eye-spot organism was not known to occur on any host other than sugar cane. It was observed, however, that the colors associated with the napier

[1] Faris, James A. Three Helminthosporium diseases of sugar cane. Phytopath. **18**: 753–774. 1928.

[2] Bourne, B. A. Studies on the ring spot disease of sugar cane. Univ. of Fla. Agr. Exp. Stat. Bull. 267. 1934.

[3] Voorhees, R. K. Eye-spot disease of napier grass. Phytopath. **28**: 438–443. 1938.

grass eye spot, in which the margins were described as being deep-red to Bordeaux red, differ from those of the lemon-grass eye spot.

DESCRIPTION OF THE DISEASE

Typical spots on the leaves of lemon grass are shown in figure 1, A, viewed both from the upper and lower surfaces. The young spots appear at first as minute yellowish flecks with a rusty-brown center. Viewed from the under side of the leaf, when the spots become about 2 mm. wide by 6 mm. long, the length being always greatest in the direction of the leaf veins, a small oval portion of the center turns a light flesh or straw color, outside of which is a dark-purple, narrow, oval border surrounded by a somewhat oval Spanish-raisin color zone, which, in turn, is surrounded by a yellowish to flesh-color areola, is often elongated at both ends in the direction of the leaf veins. Full-size spots are approximately 1.5–2 mm. wide by 4–10 mm. long. It often happens that 2 or more spots will coalesce either laterally or longitudinally and the yellowish to flesh-color areolae will then merge, forming an irregular blotch with the individual darker oval zones still in evidence. Sometimes, spots commencing at the leaf margin will spread along the same for several centimeters, the edge toward the midrib being yellowish to flesh-color and the outer edge a Spanish-raisin to dark-brown.

A cross section of the leaf through the center of a spot is shown in figure 1, B, and serves to illustrate the shrivelling of the tissues in the central portion, which becomes necrotic. The fungus mycelium was found to be both intra- and intercellular.

RESULTS OF ISOLATION EXPERIMENTS

Portions of leaves with typical spots, when placed in a damp chamber for 24 hours at room temperature, were found invariably to develop conidiophores and conidia of a species of *Helminthosporium.* Numerous tissue plantings in nutrient agar (corn-meal and lemon-grass leaf-decoction agars used) were made; almost 90 per cent of them gave rise to pure cultures of *Helminthosporium,* all of which appeared identical in their growth characters.

INOCULATION EXPERIMENTS

Young lemon-grass plants were secured by cutting back healthy shoots below the apical bud and severing the stem about 4 in. below this. These stems with dormant buds were then washed thoroughly and immersed for 10 min. in 1–1000 $HgCl_2$ solution before planting in sterile soil previously prepared in pots. After planting, the pots were covered with a bell jar and grown in the greenhouse. Many cultures prepared in this way developed healthy plants free from leaf spots. These were then used for inoculation with pure cultures of the isolated *Helminthosporium*, without wounding the leaves. In 48 hours, minute spots appeared, and, after 7 days, typical eye-spot lesions developed. Figure 1, C, shows a typical spot developing in an infection court after 7 days. Many reisolations of the fungus were made

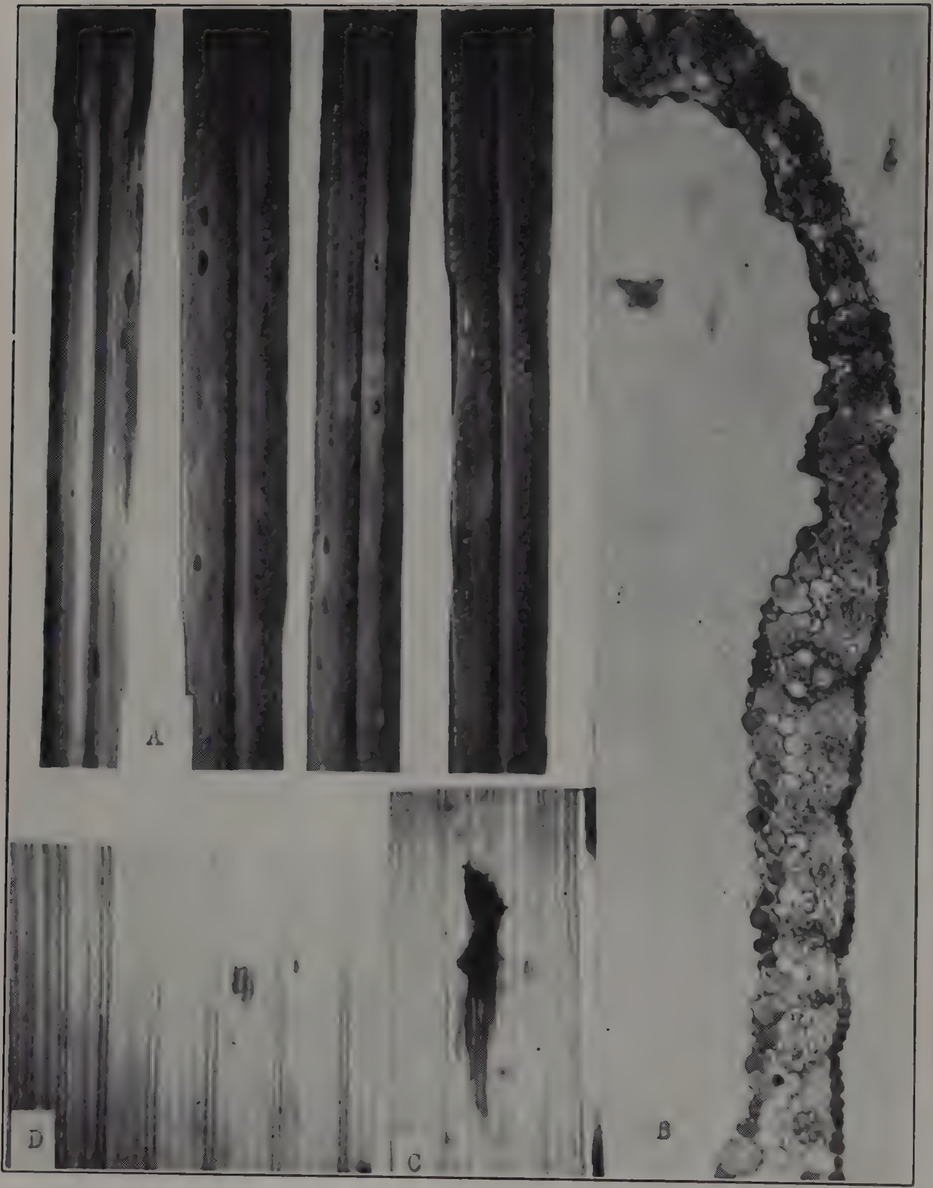

FIG. 1. A. Typical eye spots on leaves of lemon grass (*Cymbopogon citratus*), both upper and lower surfaces. B. Cross section of lemon grass leaf through eye-spot lesion, showing necrosis of center of spot. × 102. C. Infection court of lemon grass leaf inoculated with piece of mycelium from *Helminthosporium* isolated from lemon grass, after 7 days. D. Eye spots developing on Otaheite sugar-cane leaf 7 days after inoculation with lemon-grass *Helminthosporium*.

from typical lesions produced artificially, and in all cases were identical with that used for inoculation. No control cultures developed any leaf spots.

Pure-culture inoculations also were made with the lemon-grass *Helminthosporium* on the leaves of Otaheite sugar cane growing under aseptic conditions, as already described by the writer.[4] Within 24 hours after inoculation with the fungus, innumerable spots were found clearly visible. Figure 1, D, shows typical spots 7 days after inoculation with spores. Within 2 weeks, typical sugar-cane eye spots, as previously described,[4] were produced. These indicated clearly that the lemon-grass fungus is capable of producing spots on the leaves of sugar cane that are identical with those produced by *H. ocellum* Faris, which Stevenson and Rands[5] now consider to be the same as *H. sacchari* (v. Breda de Haan) Butler. The fungus was reisolated from the cane leaves and identified as being the same as that originally used for inoculation.

In order to make certain that the lemon-grass eye-spot and sugar-cane eye-spot fungi are the same organisms, a pure culture of *Helminthosporium ocellum* was isolated from the leaves of the sugar cane variety B. 3124 at Clewiston, Florida. Using this sugar-cane *H. ocellum* for inoculation experiments on lemon-grass leaves in the same manner as those already described for inoculations with the lemon-grass *Helminthosporium,* it was definitely determined from repeated trials that the sugar-cane *H. ocellum* produced spots typical of lemon-grass eye spot.

IDENTIFICATION OF THE CAUSAL FUNGUS

Pure cultures of the *Helminthosporium* from lemon grass were grown on sterile lemon-grass leaves and corn-meal agar alongside of pure cultures of *H. ocellum* isolated from B. 3124 sugar cane. Spore measurements and other morphological characters were compared, and these studies showed that the two fungi could not be distinguished from one another.

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GERMINATION EXPERIMENTS WITH UREDIO– AND TELIOSPORES OF TRANZSCHELIA PRUNI–SPINOSAE DISCOLOR

JOHN C. DUNEGAN AND CLAYTON O. SMITH[1]

(Accepted for publication July 20, 1940)

Temperature relations for the germination of the urediospores of *Tranzschelia pruni-spinosae discolor* were studied at 10, 15, 20, 23, 25, 30, and 35.5 degrees C. The urediospores were sown on water agar and incubated at the above temperatures for 3 hours and then killed by exposure to formaldehyde. The length of 25 germ tubes was measured for each of the above temperatures and the averages of these measurements are given in table 1.

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[4] See footnote 2.

[5] Stevenson, John A., and R. D. Rands. An annotated list of the fungi and bacteria associated with sugarcane and its products. The Haw. Pl. Record 42: 247–313. 1938.

[1] Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Arkansas Agricultural Experiment Station, Fayetteville, Ark., and the University of California, Citrus Experiment Station, Riverside, California.

TABLE 1.—*Average length of urediospore germ tubes at end of 3 hours*

Temperature °C.	10	15	20	23	25	30	35.5
Average length in microns	95	122	161	143	120	61	0

There were only a few spores showing the beginning of germination at 30° and none germinated at 35.5° C. There was vigorous germ-tube growth at temperatures ranging from 10° to 25° C. The optimum temperature for germination is probably similar to that for growth of germ tubes, *i.e.*, 20°–23°. Goldsworthy and Smith,[2] in their study of a rust of clingstone peaches, reported that the best growth of germ tubes was obtained at about 22° C., while the optimum temperature for germination was found to lie between 13° and 26° C.

Germ tubes on the surface of agar in Petri dishes may reach a length of 750 μ. The movement, by air currents, of these long germ tubes was observed under the microscope.

The effect of various environmental conditions on the longevity of the urediospores was studied experimentally at Fayetteville, Arkansas, from 1934 to 1938. The results of these experiments are summarized in table 2.

TABLE 2.—*Effect of various treatments on viability of urediospores of Tranzschelia pruni-spinosae discolor*

Host	Source of material	Treatment	Number of days urediospores retained viability
Prunus persica S. & Z.	Arkansas	Leaves on ground under trees	47
P. davidiana Franch.	Georgia	Leaves in cheesecloth packets, outdoors	72
P. persica	do.	do	7
P. armeniaca L.	California	Leaves in packets indoors, at room temp.	116
P. communis Fritsch.	do.	do.	177
P. domestica L.	do.	do.	112
P. persica	do.	do.	116
do.	Georgia	Leaves in packets at 5° C. in an electrical refrigerator	537
P. davidiana	do.	do.	537
P. domestica	Oregon	do.	453

The results were variable, confirming Goldsworthy and Smith's[2] statement that only relative values are obtained in experiments of this type since the source and age of the material, method of gathering the spores, temperature and other factors have a pronounced effect upon the longevity of the urediospores. However, the results of storage at 5° C. in an electrical refrigerator indicate that low temperature greatly prolongs the longevity of the urediospores.

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[2] Goldsworthy, M. C., and R. E. Smith. Studies on a rust of clingstone peaches in California. Phytopath. 21: 133–168. 1931.

During the period February 23 to March 14, 1939, teliospores of the *discolor* variety from overwintered leaves of *Prunus communis,* collected in California, were sown on water agar on 6 occasions. Promycelia, basidia, and spores developed within 24 hours in each experiment. In these tests the promycelium developed from either cell of the teliospores. Thus it was evident, at least with material from *P. communis,* that the smaller size and distorted shape of the basal cell did not render it nonfunctional, a possibility suggested in a previous paper.[3]

The one outstanding difference noted between the germinating teliospores of the *discolor* variety from *Prunus communis* and the *typica* variety from *P. serotina* Ehrh. was the location of the germ pore in each cell. In teliospores of the *discolor* variety the pore in each cell was near the septum between the two cells. In teliospores of the *typica* variety the pore in the upper cell was apical, while that of the basal cell was near the pedicel. As the result of this difference in the location of germ pores, the promycelia of the *discolor* variety emerged from the central portion, while those of the *typica* variety emerged from the ends of the spore (Fig. 1, A and B).

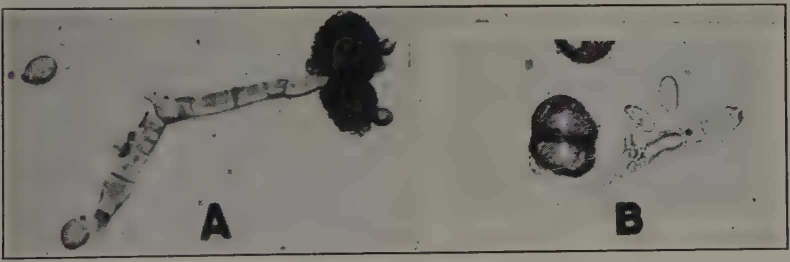

FIG. 1. A. Promycelium produced by overwintered teliospore of *Tranzschelia pruni-spinosae discolor.* B. Promycelium produced by overwintered teliospore of *T. pruni-spinosae typica.* Both × 360.

An effort was made to locate the pores in teliospores of various herbarium specimens of the *discolor* variety by bleaching the spores with a commercial preparation containing sodium hypochlorite, sodium hydroxide, and sodium chloride. The bleached spores were treated with various stains, but the final results were inconclusive, as the pores could not be distinguished with certainty in any of the tests.

Aside from the difference in the location of the germ pores, the only other difference noted was in the size and shape of the basidiospores. Those produced by the *discolor* variety were almost globose, measuring 8.0 to 8.5 μ long and 6.0 to 6.5 μ wide, while those produced by the *typica* variety were reniform, 12.5 to 16 μ long and 5.5 to 6.5 μ wide.

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[3] Dunegan, J. C. Germination experiments with overwintered teliospores of *Tranzschelia pruni-spinosae.* Phytopath. 26: 72–78. 1939.

BACTERIAL LEAF SPOT OF GARDENIA

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(Accepted for publication July 18, 1940)

In early October, 1938, the junior writer noted a severe spotting of the foliage of Belmont gardenias growing in a greenhouse at New Brunswick, N. J. At the time of observation more than 100 potted 1-year-old plants showed the spotting, principally on the lower leaves.

The spots were ovoid to circular, and varied in size from pin-point, water-soaked dots up to spots of 6 mm. in diameter (Fig. 1). The centers of the larger spots were brown to reddish-brown and were usually surrounded by a narrow water-soaked margin. The formation of several spots on a leaf resulted in yellowing and premature defoliation. Throughout late fall the spots increased in numbers and spread upward on the plants. As a result of the severe spotting and yellowing, more than half the foliage had dropped from the plants by January, 1939.

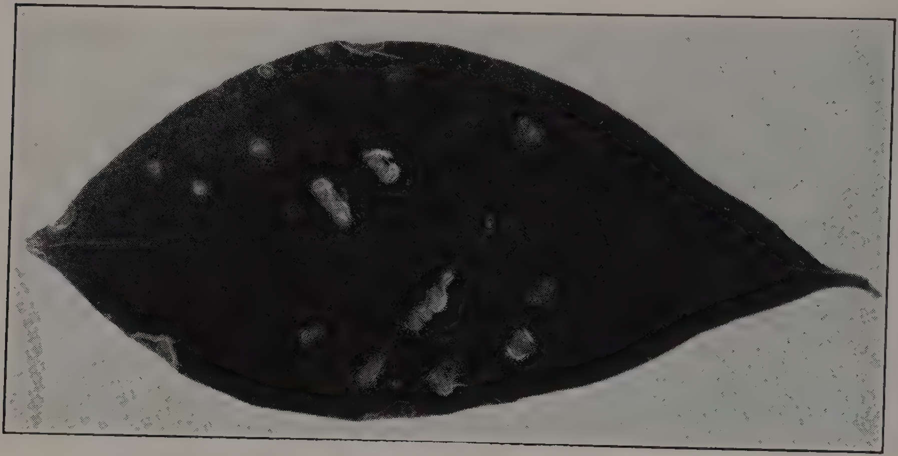

FIG. 1. Bacterial leaf spot of gardenia (natural infection).

Several extended periods of cold, cloudy, wet weather preceded the severe outbreak under observation. During these periods the humidity in the greenhouses was unusually high and the leaves remained wet for several days. The common mealy bug (*Pseudococcus citri* Risso) was the only pest prevalent on the plants.

Apparently, very little difference in varietal susceptibility exists, since the disease was noted to be equally severe on strains of Belmont, Veitchii, Mystery, Hadley, Piersons, and on the botanical variety *Gardenia radicans*.

Gardenias have been grown for a number of years in the houses where the present outbreak occurred. The grower initiated a practice, new this year, that may explain how the organism entered his greenhouse. In the spring of 1938, cuttings of several gardenia varieties were obtained from the South. After the cuttings were rooted, an occasional spotted leaf was observed that eventually turned yellow and either dropped or was picked off. The rooted cuttings were then potted and placed on benches adjacent to northern-grown Belmont and Veitchii gardenias. Circumstantial evidence, at least, points to the possibility that the organism was introduced on the southern stock. The senior writer also has seen what he believes to be the same disease on gardenias at Orlando, Florida.

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[2] N. J. Agr. Expt. Station, New Brunswick, N. J.

Microscopical examinations were made of many lesions on the leaves of the diseased gardenias and the number of bacteria found in the necrotic tissue appeared to be so great and so constant that it seemed likely they were the cause of the disease. Fungi were not observed. Dilution plates yielded the same organism in all cases and for both writers. In appearance the colonies were fast-growing and whitish, and, when plated on beef-extract-peptone agar, produced a dark coloration throughout the medium.

Numerous inoculation experiments were made on gardenias in the greenhouse with this bacterium and with several other species that appeared at various times in dilution plates. The organism isolated constantly was the only one that gave infection. The plants used in the experiments were received from the Department of Floriculture and were of the variety Veitchii, which, according to Bailey, belongs to *Gardenia jasminoides* Ellis.

There were certain environmental and other conditions, however, necessary before lesions developed after inoculation. A moist atmosphere was essential for the development of the lesion beyond a millimeter or so in diameter. Very little infection was obtained on plants placed on beds in houses where the humidity was low. When the plants were kept in moist chambers and sprayed with a water suspension of the bacteria, infection always occurred. What part temperature plays is not known, since all the plants were kept in houses 70° or above. While a lower temperature might check the disease, it would not be favorable for the growth of the host plant.

Infection occurred readily on the small tender leaves, but only very small lesions occurred on the older leaves. When spots appeared on the very young leaves, defoliation as a rule resulted. Infection was obtained more easily when the leaves were injured by pricking with very fine pointed needles. The incubation period was approximately a week, after which, microscopical examination of the lesions showed large numbers of bacteria. Reisolations were made readily.

In describing the pathogen, 8 isolates were used, 4 from the New Jersey material and 4 were isolates from artificially inoculated plants. All 8 isolates behaved similarly.

The description of the pathogen in culture follows.

The organism is a narrow rod, occurring in pairs and occasional chains. At times it is slightly curved. On beef-extract-peptone agar at 27° C. cells from a 24-hr. culture gave the following measurements: 2.4 μ (1.4 – 3.5) ×

.75 μ (.50 – 1.0). Bacteria from recently isolated cultures appear to be somewhat larger. The cells are Gram-negative and motile with 1 to 2 polar flagella. Motility was not observed under all conditions. In all cases the flagella, when stained, were more delicate than the flagella on *Phytomonas campestris* (Pammel) Bergey *et al.*, which were stained at the same time.

On beef-extract-peptone agar, at 27° C., growth is fairly rapid, white at first, but soon becomes very dirty in appearance and the medium becomes a dark brown. The colony is viscid in consistency. Bouillon becomes turbid with a pellicle, and the medium turns dark. In Litmus milk, a soft curd and pellicle are formed. The milk clears in zones and the litmus is reduced. Gelatin is liquefied. Hydrogen sulphide and indol are not produced. Nitrates are reduced to nitrites. Starch is not hydrolyzed. The following carbon compounds are utilized with the production of acid; dextrose, galactose, xylose, rhamnose, sucrose, and maltose, mannitol, glycerol, and salicin. The following organic acid salts are utilized with an alkaline production: citric, malic, malonic, succinic, tartaric, and hyppuric.

The brown pigment produced by the pathogen in the various media was considered at first to be a tyrosine reaction, since it was not formed when a synthetic medium with inorganic nitrogen was used. Furthermore, when dextrose was added to an organic medium there was no discoloration indicative of this reaction. Nevertheless, when .05 per cent tyrosine was added to an inorganic, synthetic solution as the only carbon source, the solution remained uncolored, although an extremely luxurious growth was obtained. The heavy sediment of bacteria in the bottom of the tubes, however, was dark-colored.

As far as the writers are aware no bacterial pathogen has been described on gardenias. In culture the organism is similar to *Phytomonas solanacearum* (E. Smith) Bergey *et al.*, although certain minor characteristics are different. Furthermore, infection was not obtained on potato. The organism appears to be new to literature, and, although only a weak pathogen, it belongs, no doubt, with the plant pathogens. The name **Phytomonas gardeniae**, sp. n. is proposed for it and it should be placed in the *Pseudomonas* group of this genus despite the fact that a green, fluorescent pigment has not been observed. All other characteristics point to this position.

A NEW ASCOCHYTELLA ON PENTSTEMON FROM CALIFORNIA

ANNA E. JENKINS

(Accepted for publication July 20, 1940)

A severe spotting of young stems of *Pentstemon spectabilis* Thurb. growing wild in San Antonio Canyon, near Claremont, Los Angeles County, California, was discovered in March, 1939, by Shear and Fawcett while on a mycological collecting trip in that canyon. The highly colored raised lesions (Fig. 1, A) suggested those caused by *Elsinoë*. Fruit bodies were absent, except for a few dark pycnidia (Fig. 1, B), with colored 2-celled spores, visible only after careful examination of the herbarium material collected.

Tissue cultures were made at Washington from the young lesions as a further means of determining the cause of the disfiguring disease. A number of plantings were made on potato-dextrose agar. All cultures yielded promptly a uniform type of growth. This was of more rapid development than *Elsinoë,* and, otherwise, not of the nature of this genus. The cultures were cottony and salmon-pink, and by June 12 had produced dark pycnidia corresponding to those on the young stem lesions.

On this date transfers again were made on potato-dextrose agar. These developed as before, producing the dark pycnidia, noticeable against the light background of the cultural growth. As studied by the writers, these possessed from 1 to 3 ostioles; conidia were produced in abundance. The illustration shown (Fig. 1, D) is from a mount made in July by Fawcett, in California. Pycnidia with abundant conidia also were to be seen in this set of cultures, held from July to late March in a cool temperature (refrigerator) at Washington, D. C. In these old cultures the spores, measuring from 11–21 × 4.5 × 6 μ, were commonly 1-septate, but those having 2 and 3 septa were also present (Fig. 1, C). The conidia, generally straight, were sometimes slightly curved or constricted at the septum. At the ends they were generally rounded, although in some cases one end was somewhat pointed.

note [illegible]
in cultures

Cultures transferred to potato-dextrose agar on Mar. 27, 1940, failed to produce pycnidia when observed after several weeks. On April 10, the color of the cottony growth, reading from the upper part of the slant downward, was as follows: Grayish or nearly "cartridge buff,"[1] warm white, chiefly "coral pink"[1] (base of slant) and white (agar surface below slant that had receded from glass).

Further study of the diseased *Pentstemon* by Shear revealed that infection was evidently not limited to small areas, but that the stem might be discolored and killed for 10 cm. or more. On these dead surfaces the pycnidia already described were present and were producing the 1-septate colored conidia. Their measurements here were 12–13 × 2–4 μ. Several other fungi of secondary nature were also present on the dead tissue of these older stems.

The imperfect fungus in question appears to be of the genus *Ascochytella* Died.[2] and to be undescribed. It, therefore, is here described as new under the name *Ascochytella pentstemonii,* with the following diagnosis:

✓ **Ascochytella pentstemonii,** n. sp.

Spots on stems of living plants, at first punctiform, enlarging to form spherical or irregular areas reaching 4 mm. in diameter, larger by confluence, purple at first, becoming white or pale at the center, the purple color persisting in the somewhat raised border, entire stem sometimes discolored for 10 or more cm.; pycnidia superficial or nearly so, dark colored 110 μ in diameter and 55 μ high, or larger, in culture reaching 400 μ in diam.; membranous wall about 10 μ thick; bearing conidia abundantly (method of attachment not seen), usually straight but occasionally curved at ends rounded or sometimes pointed at one end, typically 1-septate, less often 2 or 3 septate (observed in culture), generally

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[1] Ridgway, R. Color standards and color nomenclature. 43 p. Washington, D. C.

[2] Diedicke, H. Die Abteilung Hyalodidymae der Sphaeroiden. Ann Myc. 10: 141. 1912.

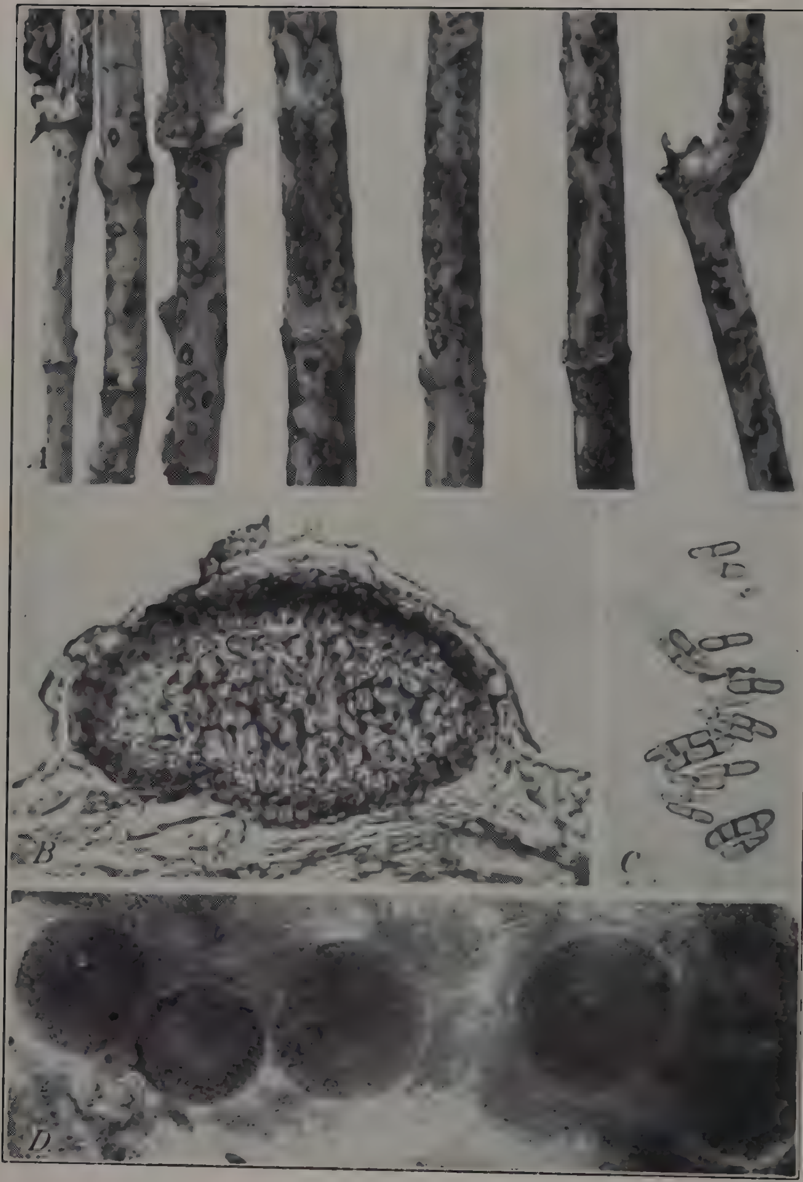

FIG. 1. A. Lesions on stems of *Pentstemon spectabilis* on which the *Ascochytella* was fruiting sparsely and from which the organism was isolated. Specimen from San Antonio Canyon, vic. Claremont, Los Angeles Co., California, Mar. 16, 1939, C. L. Shear and H. S. Fawcett. × 1. B. Pycnidium, filled with 2-celled conidia, from small lesions on green stem. × 500. C. Conidia from an old culture. × 500. D. Pycnidia produced from a culture 14 weeks old. × 60. Photographs by M. L. F. Foubert.

not constricted at the septum, but instances of slight constriction observed, clear brown, 12–13 × 2–4 μ, as observed in culture at first hyaline, and here attaining a larger size, viz., 12–21 × 4.5–6 μ.

Maculis plerumque elevatis, centro albo et margine purpureo, usque 4 mm. diam., saepe numerosis, coalescentibus, et caulem totum afficientibus; pycnidiis superficialibus vel erumpentibus, fuscis, membranaceis, 110 μ diam. et 55 μ altis vel majoribus, in culturis pluri-ostiolatis et 400 μ in diam.; conidiis fuscis, 1-septatis, 12–13 × 2–4 μ, in culturis interdum 1–3-septatis, 12–31 × 4.5–6 μ.

HOST AND DISTRIBUTION AND SPECIMENS EXAMINED

On stems of living *Pentstemon spectabilis* growing wild and apparently causing the small stem cankers or larger discolored areas on which it grows: California, Los Angeles Co., near Claremont, March 16, 1939, C. L. Shear and H. S. Fawcett (type), in the Herbarium of the University of California Citrus Experiment Station (No. 2050) and in the Mycological Collections of the Bureau of Plant Industry (73059); culture from this specimen also in the Mycological Collections (No. 73506). A living culture of the fungus (Jenkins 576) is also being maintained.

Since the *Ascochytella* appears to be pathogenic on the wild *Pentstemon,* which is also in cultivation as an ornamental, it would be desirable to make inoculations to prove definitely the pathogenecity of the organism. Until the present, however, such continued investigations by the writer has been impossible.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

PHYTOPATHOLOGICAL NOTES

Longevity of Teliospores of Puccinia graminis under Laboratory Conditions.[1]—Few records appear to be available on the length of time that teliospores retain their viability under laboratory conditions. Ward,[2] however, has reported, in a legend accompanying drawings of germinating teliospores of *Puccinia graminis* Pers., that some of the spores had been kept in his laboratory for 3 years before they were germinated.

In connection with hybridization studies with physiologic races of *Puccinia graminis,* it has been the practice of the writer to break the dormancy of greenhouse-produced teliospores by freezing them in cubes of ice in a refrigerator and thereafter to store them in a dry condition at a temperature of from 8° to 10° C. Through this procedure, two types of data have been accumulated: 1. Data on the length of time spores may be frozen in ice without loss of germinability; and 2. Data on the length of time spores may be kept in storage, after their dormancy has been broken, without loss of ability to germinate.

In table 1 is shown the germination of 4 lots of teliospores frozen for periods ranging from 14½ months to 2½ years. During these periods the spores were subjected to continuous frost except when the refrigerator was defrosted, about once a month for a few hours. Germination following the freezing periods was abundant in all cases. The sporidia produced by teliospores frozen for 2½ years gave rise to normal pycnia and aecia.

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[1] Contribution No. 607, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Ward, H. Marshall. Illustrations of the structure and life-history of *Puccinia graminis,* the fungus causing the "rust" of wheat. Ann. Bot. 2: 217–222. 1888.

TABLE 1.—*Germination of teliospores of Puccinia graminis following various periods of freezing*

Race tested	Period of freezing		Germination after freezing
	Years	Months	
P. gr. tritici, race 104	1	9½	Excellent
" " " 1	1	2½	"
P. gr. avenae, " 7	1	2½	Good
" *secalis*	2	6	Good[a]

[a] Good infection on barberry produced by sporidia.

In table 2 are given a number of instances in which teliospores of physiologic races of wheat stem rust and oat stem rust have germinated after long intervals of storage. Periods of storage of from 2 to 3 years apparently did not impair the germinability of the spores or the infectivity of the sporidia produced. The longest storage periods—6 years, 2½ months, for wheat stem rust, and 4 years, 4 months, for oat stem rust—did not noticeably affect germinability but did cause an apparent diminution of the pathogenic vigor of the rust on barberry. Some of the pycnial pustules produced by the wheat stem rust following this long period of storage were normal in appearance, but many were quite small and bore only a scanty exudate of pycnial nectar. The apparent effect of ageing of teliospores on the pathogenicity of the oat stem rust referred to above has been discussed elsewhere.[3]

TABLE 2.—*Germination of teliospores of Puccinia graminis following various periods of storage at 8°–10° C.*

Race tested	Period of freezing	Period of storage after freezing		Germination following storage
	Months	Years	Months	
P. gr. tritici, race 9	7½	6	2½	Good[a]
" " " 17	8½	2	10	Slight[b]
" " " 57	8	2	10	Fair[b]
" " " 120	8	2	10	Fair[b]
" " " 57	10	2	8	Good[b]
" " " 52	10	2	8	Fair[b]
P. gr. avenae, " 3	5	4	4	Excellent[c]
" " " 8	1	2	9	Excellent[b]
" " " 8	12	2	8	Fair[b]
" " " 6	9	2	7	Slight[b]
" " " 6	9	2	7	Slight[b]
" " " 10	8½	2	1½	Fair[b]

[a] Rather weak infection on barberry produced by sporidia.
[b] Good infection on barberry produced by sporidia.
[c] About half of sporidia produced normal infections, half produced necrotic flecks only.

Although the extreme longevity of teliospores under the conditions specified above is not known, it is clear that their viability may be preserved for relatively long periods by keeping them frozen or by storing them in a dry condition at low temperatures.—T. JOHNSON, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

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[3] Johnson, T., and Margaret Newton. Crossing and selfing studies with physiologic races of oat stem rust. Can. Jour. Research C, 18: 54–67. 1940.

Bacterial Wilt of Carnation.—Wilting and death of carnation plants has been observed in a glasshouse in Spokane, Washington. Isolation cultures of the organism and inoculation tests have shown the disease to be caused by a bacterium that appears to be distinct from any previously described plant pathogen. The production of greyish-green foliage, followed by yellowing and death of the plants, similar to the symptoms commonly associated with Fusarium wilt, is characteristic of the disease. Yellow streaks of frayed tissue in the vascular areas extending 12–24 inches up the stems of affected plants are distinguishing symptoms.

The disease has been observed in the commercial glasshouse on Kathryn, Melrose, and King Cardinal varieties causing the death of 30, 15, and 5 per cent of the plants, respectively. Inoculation tests show that Chief Kokomo, Giant Laddie, Joan Marie, King Cardinal, Matchless, Maytime, My Love, Patrician, Pink Spectrum, Puritan, Sophelia, and White My Love are susceptible to infection. The period of incubation is longer and the damage less severe on Giant Laddie and Joan Marie than with the other varieties.

The disease appears most damaging during the warm weather of fall and spring. The minimum period of incubation has been 30 days following inoculation into punctures at the base of the stem. Cuttings taken from plants previous to the appearance of symptoms have transmitted the organism. Since the disease was observed on the Kathryn and Melrose varieties in the glasshouse in question, 2 years after the original stock was purchased, and the disease has not been observed in any other glasshouse in Washington, it is probable that it was introduced into the Spokane area from another section of the country. Determinative studies on the causal organism are being made by W. H. Burkholder of Cornell University.—LEON K. JONES, Division of Plant Pathology, State College of Washington, Pullman, Washington.

REPORT OF THE COMMITTEE ON BIOLOGICAL ABSTRACTS AND THE UNION OF AMERICAN BIOLOGICAL SOCIETIES

Before the current war Biological Abstracts had already successfully met its crisis and was fast moving toward financial independence through subscriptions, even though priced far below any comparable service in its field.

It was rendering, as a cooperative, non-profit enterprise, a gratifyingly prompt service with all indexes up to date. In 1939, 18,000 abstracts were published from 1,150 publication sources. The separate Plant Science Section "D" contained 4,302 abstracts from 682 sources, including 648 phytopathological abstracts from 204 sources, as well as world research in plant physiology, anatomy, ecology, taxonomy, biometeorology, horticulture, agronomy, and forestry.

In 1940, however, the war brought cancellations of many foreign subscriptions. More will undoubtedly follow. To meet this new crisis, the budget was cut. Mr. H. I. Anderson, the new Business Manager, made energetic efforts to bring in new subscriptions. Societies continued their aid. The results are distinctly encouraging. The reserve fund will hardly be touched, thus providing a backlog for 1941. In spite of the curtailed budget, over 17,000 abstracts and the usual indexes were issued in 1940. Promptness was continued. Cooperation is needed, however, to raise subscriptions to the self-sustaining level.

Your Committee suggests that Society members can help most by local canvassing. Are the main library and departmental reading rooms provided with enough copies for future, as well as present, reference? Are nearby teachers' colleges, junior colleges, public and high school libraries supplied? Do local industries with biological interests realize the time saved at low cost through use of Biological Abstracts? Your Committee will appreciate cooperation in making this important service available wherever needed.

HOWARD P. BARSS,
Chairman.

December 27, 1940.

BOOK REVIEW

DICKSON, JAMES G. *Outline of Diseases of Cereal and Forage Crop Plants of the Northern Part of the United States.* 259 pp. Burgess Publishing Co., Minneapolis, Minnesota. 1939. $3.00.

This book, an outline pertaining exclusively to diseases of field crops, is the first to bring together in one place information and literature citations that, heretofore, were available only in bulletins, scattered scientific journals, and in text or reference books. The index is well-done, although it might be improved by including all the common synonyms of the pathogens given in the text. The book is reproduced in mimeoprint and contains neither photographs nor other illustrative material.

The text is divided into two sections: Part 1, diseases of cereal crops (including flax); and Part 2, diseases of forage crops. The first part (197 pp.) includes chapters on diseases of barley, corn, oats, rye, sorghum and millet, wheat, and flax; while the second part (42 pp.) is divided into 3 chapters dealing with diseases of alfalfa and sweetclover, clover, and soybean. Diseases are considered in the following sequence: nonparasitic, virus, bacterial, phycomycetous, ascomycetous, hypomycetous, and basidiomycetous. This arrangement necessitates considerable repetition when one disease occurs on several hosts. Only the major diseases have been discussed, and the number affecting a crop varies from 8 to 23. About 30 per cent of the book is devoted to information and citations on 23 different diseases of wheat.

The more important diseases are discussed from the following standpoints: hosts, geographic distribution, economic importance, description of symptoms, pathological histology, causal organism, seasonal development, and control measures; while the less important ones are presented only briefly. Keys for the determination of physiologic races of many of the important pathogens also are included. The pertinent literature is listed at the end of the section on each disease. More than 2300 literature citations are given.

In some cases, direct references to the author or his work would be helpful, especially, for those who wish to consult the original articles; often a more complete statement would add materially to its value. A wider separation of the diseases within a chapter would make the text more readable. Nevertheless, these criticisms and the few minor errors do not detract noticeably from the quality of the book, which the author intended only as an outline. One is impressed with the author's thorough familiarity with most of the diseases and his understanding of the disease problems confronting pathologists. A book of this type has long been needed, and it will be a valuable guide and reference book for students, pathologists, and plant breeders.—J. J. CHRISTENSEN.

TECHNIQUES FOR APPRAISING AIR–BORNE POPULATIONS OF MICROORGANISMS, POLLEN, AND INSECTS[1]

The Committee on Apparatus in Aerobiology, National Research Council[2]

(Accepted for publication September 10, 1940)

INTRODUCTION

The cholera epidemic of 1848 and, later, the controversy over the question of spontaneous generation stimulated an early interest in the microbial population of the air, so that many of the pioneer studies of this subject were made by workers in the latter half of the 19th century whose objectives were the elucidation of the problems relating to the germ theory of disease and the origin of life. During the present century the study of the microbial and insect population of the air has been taken up by several groups of investigators with different objectives, somewhat different backgrounds, and to a certain extent familiar with different literature. With regard to primary interests these workers seem to fall naturally into the following groups: 1. Sanitary air analysis in ventilation studies of hospitals, schools, industries, etc. 2. Air-borne-pollen dissemination. 3. Microorganisms in the upper air. 4. Epidemiology of plant diseases. 5. Insect population and migration in the air.

The purpose of this paper is to bring to the attention of investigators in each of these fields techniques that have proved useful in related fields. Consideration is given principally to methods of catching and counting airborne material. Laboratory methods are described only when they have a direct bearing on population estimates.[3]

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[1] Approximately one-half the cost of publication of this paper was borne by the National Research Council.

[2] This committee is a subcommittee of the Committee on Aerobiology of the National Research Council, Washington, D. C. Its membership is as follows: P. N. Annand, D. M. Little, W. M. Lockhart, B. E. Proctor, W. F. Wells, R. P. Wodehouse, and E. B. Lambert, Chairman. The different sections of the paper were prepared by the following writers: Sanitary Air Analysis, W. F. Wells; Air-borne Pollen Dissemination, R. P. Wodehouse; Microorganisms in the Upper Air, B. E. Proctor; Insect Population and Migration in the Air, P. A. Glick; Germ Theory of Disease, Epidemiology of Plant Diseases, Summary and Conclusions, E. B. Lambert. The writers are grateful to Captain Harry Armstrong and to Dr. D. M. Little for collating material preliminary to the preparation of this paper. Since this sub-committee report is the first publication emanating from the committee, the writers take this opportunity to express their appreciation of the large part played by the late Fred C. Meier in inaugurating the entire program of the committee. They are also pleased to acknowledge the material aid furnished by access to Mr. Meier's bibliographic card file.

[3] Although a consideration of methods of determining industrial dusts is outside the scope of this paper, the field has much in common with aerobiology. A summary of the methods used and an entrance into the literature on this subject may be found in: Bloomfield, J. J. and J. M. Dallavalle. The Determination and Control of Industrial Dusts. Pub. Health Bull. 217. 1935.

[Volume 31, Number 3, March, 1941]

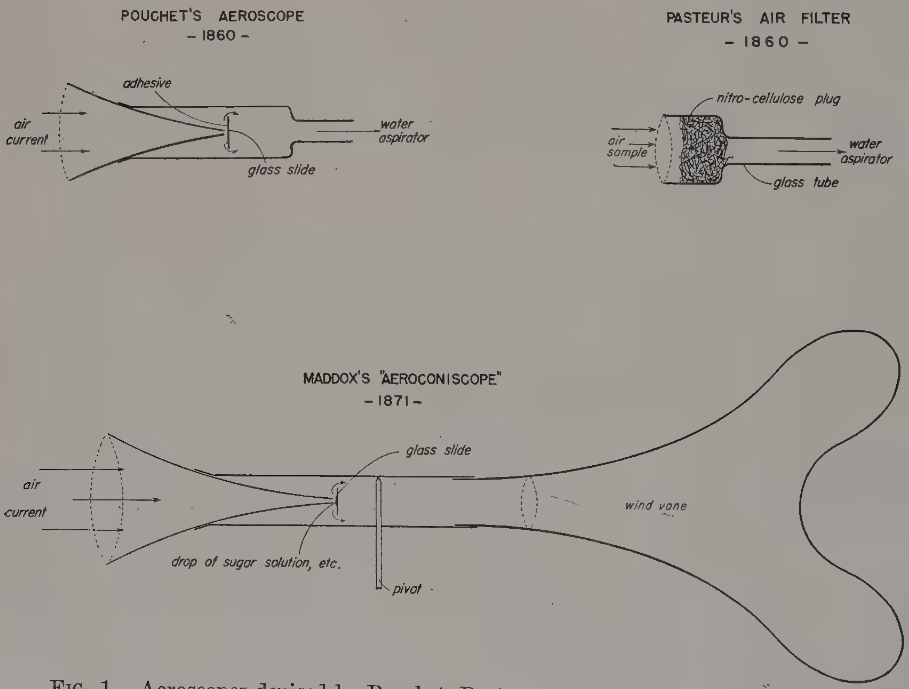
GERM THEORY OF DISEASE AND ORIGIN OF LIFE

In attempting to solve these problems by studying the microscopic population of the air, investigators devised techniques that included the use of adhesive-coated glass slides, air washers, impinging aeroscopes, and air filters.

Hogg (46) cites Osborne in 1854 as the first to catch "germs and spores of fungi" with slips of glass coated with glycerine.

Ransome (69) was one of the earliest to filter air by drawing it through distilled water. He described his method as follows: "In 1857, glass plates covered with glycerine had been exposed in different places and examined microscopically." . . . "During a crowded meeting . . . air was drawn for two hours through distilled water, and the sediment examined after thirty-six hours."

Pouchet (65) utilized the principle of the glycerine-coated slide in the construction of his aeroscope, shown in figure 1. His apparatus had many points in common with present-day practice. By means of a water aspirator he drew measured volumes of air through a funnel-shape tube onto a glass slide coated with adhesive. At the point where the air discharged onto the glass slide the tube was restricted to 0.5 mm. in diameter and ended about 1 mm. from the slide. Thus, most of the particles that adhered to the slide were in a space about 2 mm. in diameter directly opposite the small opening of the funnel.


POUCHET'S AEROSCOPE
– 1860 –

PASTEUR'S AIR FILTER
– 1860 –

MADDOX'S "AEROCONISCOPE"
– 1871 –

adhesive
air current
water aspirator
glass slide
nitro-cellulose plug
air sample
water aspirator
glass tube
glass slide
air current
wind vane
drop of sugar solution, etc.
pivot

FIG. 1. Aeroscopes devised by Pouchet, Pasteur, and Maddox for their pioneer work on the distribution of microorganisms in the air.

Maddox (52), later, Cunningham (20) and Airy (2), modified Pouchet's aeroscope by pivoting it in a horizontal position and adding a tail to keep it turned toward the wind, like the pointer of a wind vane, shown in figure 1. They placed drops of water and different plant and animal decoctions on the glass slide opposite the stream of air. By this means they germinated and cultured spores of many different microorganisms. Cunningham's drawings clearly illustrate the presence of bacteria, nematodes, fungi, and pollen grains on his slides.

Pasteur (61) used a nitrocellulose filter (Fig. 1). He described his apparatus in 1860 as follows: "By means of a continuous water aspirator I pass outside air through a small cotton plug of the modification of cotton which is soluble in alcohol and ether. The cotton arrests a part of the solid particles contained in the air. It is then dissolved in a small test tube with an alcohol-ether mixture and allowed to stand for 24 hours. When all the particles have settled to the bottom it is easy to decant the upper liquid without disturbing the sediment if one separates each decanting by a period of standing of from 12 to 24 hours. Finally, the suspended particles are poured onto a glass slide where the solvent evaporates rapidly. It is then easy to examine the particles under the microscope and submit them to various reagents." His method, of course, killed the microorganisms, but was effective in demonstrating the presence of spores in the air.

Tyndall (83) used dust-proof chambers, coated on the inside with glycerine and fitted on 3 sides with windows to obtain the "optically pure" air, which he used in experiments and demonstrations to disprove spontaneous generation. He constructed 50 of these boxes.

The best summary of the work of this group may be found in the paper published in 1873 by Cunningham (20). He reported his work in great detail, illustrated his findings with beautiful drawings, and gave a brief outline of the work of each of his colleagues.

SANITARY AIR ANALYSIS

In this field the techniques most extensively used have been modifications of the following methods: 1. Filtering air through granular material. 2. Bubbling air through sterile water. 3. Centrifuging air onto media. 4. Horizontal exposure of agar plates.

Methods of examining the air used by earlier workers were summarized by Macdonald (53) in 1883. He called attention to the aeroscopes of Pouchet and Maddox and described the experiments of Chaumont in which air is carried through a succession of bottles of pure distilled water.

Frankland (34), in 1887, devised a volumetric air filter consisting of a small tube $\frac{1}{4}$ inch in diameter by 5 inches long containing glass wool and granulated sugar as filter material. Samples of air were pulled through this filter by means of a 10-liter-bottle aspirator. Buller and Lowe (7) made extensive comparisons between the bacterial counts obtained with the Frankland filter and by exposure of agar plates. They described their methods in detail. In these studies counts were made every day for an entire year.

Petri (63) designed a sand-filtration tube in 1888, which was for many years the standard quantitative method of measuring bacterial content of air. The Petri apparatus consisted of a glass tube 9 cm. long and 1.6 cm. in diameter, filled with two columns of sand separated from each other and held in place by wire gauze. Definite volumes of air were drawn through the sterilized filter tube. The sand was then transferred to sterilized gelatine where it was shaken up and the resulting mixture plated out in Petri dishes.

Soper modified the Petri technique by passing the air successively

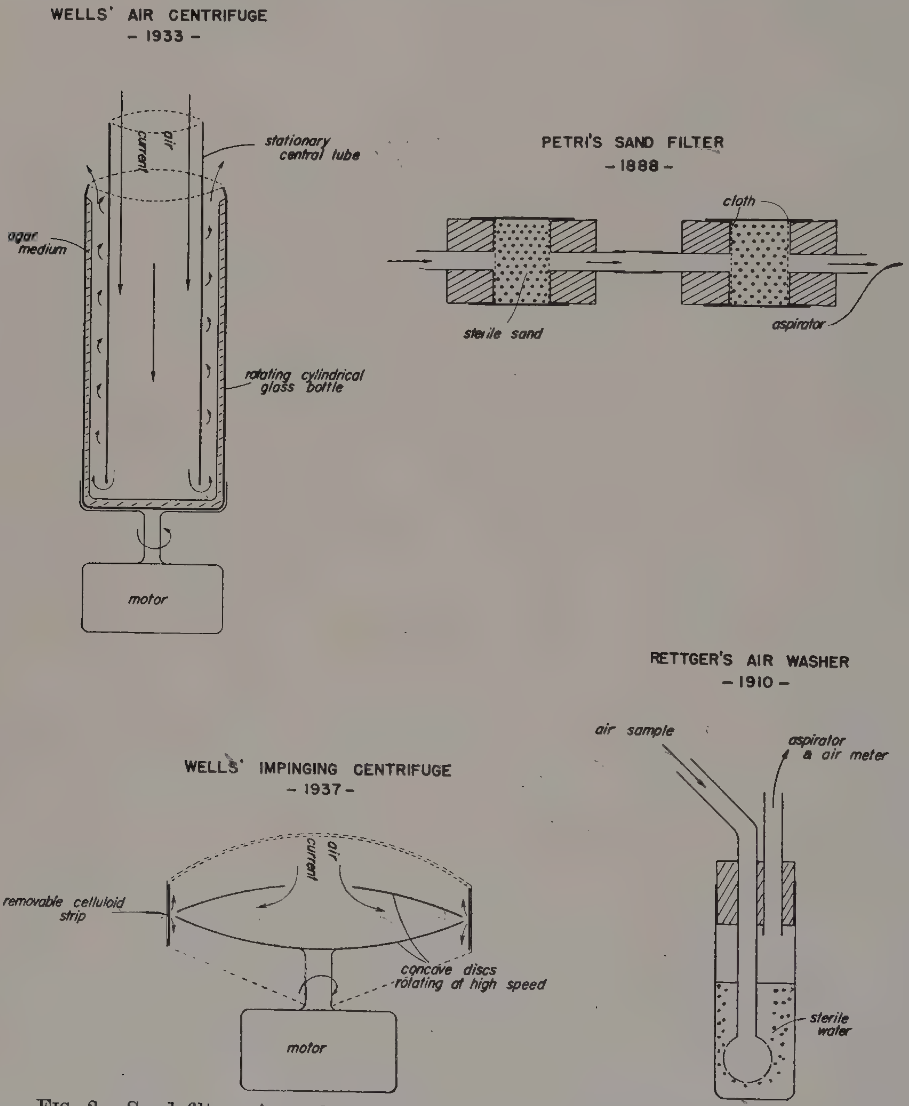

WELLS' AIR CENTRIFUGE
- 1933 -
air current
stationary central tube
agar medium
rotating cylindrical glass bottle
motor
PETRI'S SAND FILTER
- 1888 -
cloth
sterile sand
aspirator
RETTGER'S AIR WASHER
- 1910 -
air sample
aspirator & air meter
sterile water
WELLS' IMPINGING CENTRIFUGE
- 1937 -
air current
removable celluloid strip
concave discs rotating at high speed
motor

FIG. 2. Sand filter, air washer and air centrifuge developed by Petri, Rettger, and Wells, for use in intramural sanitary air analysis. The Wells Impinging Centrifuge, shown in the lower left-hand corner, was devised for determining the pollen and fungus-spore content of the air.

through two sand filters, washing the sand with sterile water or salt water, and subsequently plating out the bacterial suspensions. As illustrated in figure 2, this is essentially the standard method adopted in 1910 by a committee consisting of Winslow, Richards, Soper, Thomas, and Weinzirl (92). They recommend the following apparatus and procedure:

"Standard Method for Enumerating Bacteria in Air (Filtration method of Petri). The filter tubes, etc. The filter tubes are of glass, 1½ cm. in diameter and 10 cm. long. In the end of each is placed a perforated cork stopper through which a glass tube 6 mm. in diameter is passed. The filter consists of a layer of sand, which has been passed through a 100 mesh sieve, 1 cm. deep, supported by a layer of bolting cloth covering the cork. Two filter tubes are connected in tandem and a measured volume of air, 10 l. or more, is drawn through at a constant rate of suction. The suction is applied by means of an aspirator of known volume, preferably one of the double or continuous type. Before using a pair of filter tubes, a test for possible leakage is made by placing the thumb over the cotton stopper and applying the aspirator; if the suction is weak or absent, the corks must be tightened or the tubes discarded. All corks should be tightened and connections wired immediately before using the filters. The collection of the sample should take from 1 to 2 minutes, per liter.

"After filtering a definite volume of air through the tubes, the sand is shaken out into 10 cc. of sterile water, thoroughly shaken, and aliquot portions plated in ordinary nutrient agar, all plates being made in duplicate. The plates are incubated at room temperature for 5 days, when final counts are made. If Petri dishes 9 cm. in diameter are employed, all plates showing a larger count than 200 colonies should be rejected to eliminate inhibitive action."

This method has been used by Winslow, Baskerville, Browne, Ruehl, and others. Clark and Gage (13) used a triple sand filter and substituted a hand pump for the aspirator in obtaining air samples.

Rettger (71) advocated in 1910 a method of filtering air that involved bubbling it through sterile water (Fig. 2). The required apparatus is simple to construct and effective.

A few years later the committee of Winslow *et al.* (92) compared the "standard" Petri-filter method with the new Rettger method and concluded that there was no appreciable difference in the accuracy of the two methods. The sand filter method was again recommended on the basis of convenience of handling in the field.

Wells (89), in 1933, devised an air centrifuge that introduced an entirely new principle in the counting of the bacterial population of the air. The Wells air centrifuge (Fig. 2) is essentially a long narrow cylinder coated on the inside with a culture medium, usually agar, and so arranged that it can be rotated on its axis at high speed, causing air to be drawn slowly through a stationary central tube and to pass out over the medium. The rapid rotation (4,000 r.p.m.) of the cylinder causes a similar rotation of the air as it passes out through the bottle. Centrifugal force developed by this rotation precipitates onto the agar almost all of the dust and microorganisms contained in the air. With this machine it is almost as simple to centrifuge samples of air as to expose agar plates, yet the results obtained are quantitative measurements comparable to those heretofore attained only by the somewhat cumbersome filtration and plating methods already described. Several operations are combined in one. The centrifuge creates and measures an air flow, precipitates the floating microorganisms, and permits colony counts without further plating.

Pincus and Stern (64), after making extensive studies of air pollution in New York City with the Wells air centrifuge, were enthusiastic in their praise of the apparatus.

Wells, Phelps, Robertson, and Winslow (90) have recently discussed rather fully the advantages and limitations of the centrifugal bacteriological procedure in air analysis.

The simple expedient of exposing Petri dishes of agar in a horizontal position has been used by many investigators, notably Saito (75, 76, 77). In many studies an air-exposure time, equivalent to exposing 1 sq. ft. for 1 min., has been adopted as routine practice. This is about a 15-min. exposure with a midsize Petri dish. Zo Bell and Mathews (97) used salt-water and fresh-water media to distinguish between organisms in sea breezes and land breezes. Clark and Gage (13) found the exposure of dry plates and subsequent pouring of the media to be more convenient than the exposure of poured plates and just as effective. Clark and Gage (13), and Wells (87) made extensive comparisons of the plate-exposure method with the sand-filter and centrifuge methods. Their data indicate that the plate method gives an entirely different result. The counts from the plates were much lower and somewhat less consistent. Counts obtained from 15-min. plate exposures are usually about 1/10 as large as those obtained from 1 cu. m. filtrations or 10 cu. ft. centrifuge runs. When there was a high proportion of light particles in the air, such as ''droplet nuclei,'' the rate of settling out of particles due to gravity was found to be comparatively slow, and the plate-exposure method was even less efficient as compared to the centrifuge or the filter methods.

AIR-BORNE POLLEN DISSEMINATION

For detecting pollen of general atmospheric distribution the methods generally employed, because of their cheapness and simplicity, are those of gravitation, in which advantage is taken of the fact that pollen grains, in common with dust particles, tend always to fall through the air at a rate depending upon their size, shape, and specific gravity.

Microscope Slides

The usual method is to expose microscope slides in a horizontal position for 24 hours. Various kinds of adhesive have been employed to catch and hold the pollen, though it is doubtful if this is important in most cases, because pollen grains are of such a magnitude that when they touch an object they stick to it. This method appears to have been first used by Blackley (4), coating the slides with glycerine. But glycerine alone is unsatisfactory in rainy or even damp weather, because of its strong tendency to absorb water. A thin layer of glycerine jelly is under ordinary circumstances a better adhesive, since it provides a medium of suitable refractive index in which to observe the grains, and presents the grains in their moist and expanded condition, but not ruptured.

Wodehouse (93, 94, 95) uses commercial glycerine jelly to which enough basic fuchsin has been added to make it as dark as red ink. This is applied in a thin, uniform layer to a circular area of a microscope slide, 18 mm. in diameter. The method employed necessitates melting the basic fuchsin-glycerine jelly in a water bath. A slide is heated gently by passing it several times through a flame, fastened on a slide turntable and centered exactly. A small camel-hair brush is dipped into the jelly, the slide spun by giving the turntable a vigorous twist, the brush touched to the slide at its center of revolution and stroked slowly outwards to the boundary of the 18 mm. circle of the slide turntable. The result is a perfectly circular area, 18 mm. in diameter, evenly covered with a thin layer of melted glycerine jelly. The slides may be stored for a month or more in suitable containers, so that a dozen or two may be made up at a time and exposed as needed.

After exposure, the slide is examined with a hand lens and grains of sand or large bits of organic material, if present, are removed with a needle, so as to permit the cover glass to settle into position. The slide is then warmed enough to melt the glycerine jelly, and a No. 0 cover glass, 18 mm. in diameter, placed over it.

Both glycerine and glycerine jelly possess one serious objection; they take up moisture so readily from the atmosphere that if the weather is showery or even excessively humid the preparations usually are spoiled. Under these conditions some kind of oily medium should be substituted. Mineral oil, boiled linseed oil, and vaseline have been used. Vaseline has been extensively used by Durham (27, 28, 29, 30), Rowe (74) and others. For best results vaseline is applied in the thinnest possible layer. A satisfactory way to prepare vaseline slides is to use a slide turntable in much the same way as for glycerine jelly preparations.

After exposure the slide is brought in and examined for extraneous material that might keep the cover from settling into place. When freed from all such, a very small drop of glycerine-alcohol-water mixture with basic fuchsin added, is applied to the center of the oil smear and the slide heated gently over a small flame, and a No. 0 cover glass added. The pollen grains are thus presented stained and in a suitable medium for examination; if the vaseline smear be sparse enough the oil would interfere but slightly.

Exposing the Slides

Many different ways of exposing the slides and of protecting them from the weather during exposure have been devised. By many, *e.g.*, Scheppegrell (78, 79), it is considered sufficient to expose the slide on any flat surface, such as a window ledge, without protection. In dry regions, where there is little rain, this is sufficient; but, under most conditions, of course, many slides are spoiled by light showers from which they could easily be protected. Almost any shelter that will shed the rain and at the same time permit free access of the air will serve. A small wooden roof about the size of that of a bird-cage raised on 4 posts about 4 inches above the slide will serve very well. Acquarone and Gay (1) devised a convenient metal shelter more or less open at all sides, and with clamps inside to hold the slide.

It has repeatedly been demonstrated that slides held in a vertical position facing the wind catch considerably more pollen than in the horizontal position—provided there is wind. Penfound and Efron (62) devised a holder for two slides mounted on a weathervane. In this, one slide remains horizontal while the other is kept constantly facing the wind. Their records showed that the vertical slide caught not only absolutely more pollen than the horizontal one, but also relatively more of some kinds than of others. La Rush (50) used 5 slides for each record, 1 horizontal and 4 in upright positions, "one facing each direction of the wind," in order to meet all exigencies of this kind.

Making use of the fact that much more pollen is thrown out of the air when made to impinge upon the slide than falls out by gravity, Duke (23) devised a pollen-catching apparatus in 1923 that consists of a small blower, of the type used for hair drying, that delivered air at the rate of approximately 40 miles per hour on a greasy slide set across the air stream at an angle of 45°. His records indicate that 5 minutes' exposure with the blower are equivalent to about 24 hours' exposure by the gravity method. This apparatus is in principle a modification of Pouchet's aeroscope described elsewhere.

Recording Pollen Counts

All methods depending upon gravity for depositing the pollen on the slides are open to the objection that no exact correlation can be established between the number of grains caught on a given area in a given time with the number of grains present per unit volume of air. Consequently, most investigators depending wholly or in part on gravity for pollen deposition report their results in terms of grains caught per unit area generally in 24 hours. The area used is usually one of convenience and is stated in the record. Wodehouse prefers to use 2.5 sq. cm., or the area covered by a circular cover glass 18 mm. in diameter; the circular cover glass is necessitated if the adhesive is spun on the slide. Some investigators use square cover glasses of varying size, reporting their counts in terms of the area covered. Others report their data in terms of a number of excursions across a field of given width at a known magnification or in terms of arbitrarily chosen unit areas, *i.e.*, sq. cm., sq. in., sq. ft., by applying the necessary factors to their counts. The methods used by various workers are outlined by Cocke (14).

Scheppegrell (79), making use of Stoke's law governing falling bodies, has developed a formula for calculating the number of grains per unit volume of air from the number caught on a given area in given time:

$$n = \frac{7 \times N}{v\, t}$$

Where n = no. per cu. yd.,
N = total number falling on 1 sq. cm.,
v = velocity of fall,
t = time of exposure.

The velocity of fall (v) he calculated from Stoke's law

$$v = \frac{2\, g r^2}{9\, K}$$

Where g = the acceleration of gravity (980 cm.).
r = the radius of the sphere,
K = coefficient of viscosity of air (0.00018).

Scheppegrell assumed that the specific gravity of pollen is 1, which appears to be very nearly correct, and that pollen grains are spherical, which is hardly ever true, though in most cases the deviation from the spherical is probably not a factor of great importance. But, he adds, "the values will be considerably higher in the case of pollen with spicules, the velocity being considerably less than the calculated value." He does not say directly what the extent of this effect is, but elsewhere, in his discussion of the "distance traversed in air currents by pollen of various size," which is also based on Stoke's law, he does say, "for spiculated pollens, add 10 to 50 per cent, according to the size and length of the spicules." Presumably, if spicules extend the range of transport of pollen grains 10 to 50 per cent, they do so by slowing the rate of fall by that amount, which is probably a fair enough estimate of the effect of "spicules" on the rate of fall of pollen grains.

Unfortunately, as pointed out by Cocke (14) in 1937, Scheppegrell, in applying Stoke's law mistakenly, used the diameter of the pollen grains instead of the radius. Table 1 shows the order of magnitude of the error in Scheppegrell's data and indicates the profound effect of the size of the grain on the value of n, which illustrates the importance of considering pollen-grain size when computing their atmospheric density from Stoke's law.

On the basis of Scheppegrell's conversion factor for pollen grains 20 μ in diameter, Durham, in 1918, and others later (22, 41, 85, 91) adopted the procedure of counting pollen from an area of 1.8 sq. cm. on slides exposed for 24 hours. These counts were frequently recorded directly as the number of grains per cu. yd. in air. In the light of Cocke's recalcu-

TABLE 1.—*Relation of the diameter of smooth spherical pollen grains to the theoretical velocity of fall and the resulting formulae for computing the number of grains per cubic yard of air (n) from the number of pollen grains gathered per square centimeter on an atmospheric pollen plate in 24 hours (N)*

Diameter of pollen in μ	Velocity of fall, foot per second (Cocke)	Formulae erroneously calculated by Scheppegrell	Formulae correctly calculated by Cocke
10	0.01	$n = 7.3\ N$	$n = 29.1\ N$
20	0.04	$1.8\ N$	$7.3\ N$
30	0.09	$0.8\ N$	$3.2\ N$
40	0.16	$0.45\ N$	$1.8\ N$

lation of Scheppegrell's formulae, as indicated in table 1, it is evident that correction factors that take into consideration the correct formulae for pollen of different diameters must be applied to these data.

Cocke (15) has shown experimentally that his conversion formulae are approximately correct for most pollen grains by comparing them with actual counts of the number of pollen grains obtained from measured volumes of air. He found that "for spiculated pollen, such as that of ragweed, the actual concentration is about one-third greater than the calculate concentration," and "for pollen with air sacs or wings, *e.g.*, pine, 50 per cent should be added to the calculated atmospheric concentration." In this paper he also furnishes a table for determining at a glance the number of pollen grains of any size per cubic yard of air from the number of grains caught on one sq. cm. in 24 hours.

Open Dish Method

A method described by Lüdi and Vareschi (51) requires exposure, without cover or protection of any kind, a shallow dish such as a Petri dish, but deep enough to avoid overflow by rain. The inside of the dish is covered with a thin layer of water and glycerine. After exposure, the pollen, glycerine and accumulated rain water are washed out with pure water and centrifuged so as to concentrate the solid material into 1 or 2 cu. cm. of liquid. Then this is boiled in 10 per cent KOH for three to five minutes to remove the cell contents of the pollen grains. The material may then be suspended in a measured volume of water and the pollen grains of a measured fraction counted. From this may be calculated the number of pollen grains falling into the dish per unit area.

Rempe's Catch Tubes

Rempe (70) in a very extensive series of investigations on the atmospheric dispersal of pollen, caught his material on cellophane coated with vaseline. For this he used small brass tubes, 14 mm. in diameter and 45 mm. long. Across each end he wrapped a thin sheet of cellophane coated lightly with vaseline. The tube was then suspended horizontally, and, when necessary, protected with a conical roof 13 cm. in diameter. These tubes, with their protecting roofs, were suspended in the crowns of flowering trees and from the tops of poles of varying heights and distances from the trees under investigation. The same tubes also were used on airplane flights suspended behind the propeller. After exposure the cellophane sheets were removed and placed face down on microscope slides so that the pollen was observed in vaseline between cellophane and glass. The counts were recorded as the number of grains caught per square centimeter of exposed surface. Rempe argues that since pollen is blown onto the stigmas of plants instead of dropping on by gravity, his tubes more closely approximate natural conditions than do the horizontal slides.

Snow and Rain

Miquel (58), in 1883, found pollen in freshly fallen snow. Busse (9) collected fir pollen in rain drops.

All the methods of atmospheric pollen analysis that depend wholly or in part upon gravity should be regarded as qualitative rather than quantitative. They serve to show quickly and easily what kinds of pollen are in the air, and, if the different sizes of each are taken into account, they show the relative abundance of each. But as an absolute measure of the amount of pollen in the air, at best, they only give a rough approximation.

Air Scrubbing

Zifferblatt and Seelaus (96) used a scrubbing tower for making pollen counts. Their apparatus, described in 1935, differs from Rettger's in that they used glass beads to break up the air bubbles as is common practice in gas analysis. An integral flow meter enabled them to measure the volume of air pulled through their apparatus. They have adopted 200 cu. ft. of air as the standard unit of sampling. The material collected was examined under the microscope or plated out in the usual manner.

Air Filtration

Erdtman (31) described in 1937 a method of accurately determining the number of pollen grains of each species in measured volumes of air (Fig. 3). His apparatus, which he calls the *Aerosol Collector,* is a small vacuum cleaner with its filter bag replaced by a plaited filter paper supported on an aluminum frame. All the air that enters is forced through the filter paper and, as it leaves the apparatus, it led through a pipe where it can be metered. Through one side of the exhaust pipe is inserted a pitot tube with a short right-angle bend turned so that its orifice faces against the flow of air. The pressure exerted in the pitot tube by the flowing air is transmitted by a rubber tube to a mercury manometer. The manometer is calibrated with the assistance of an ordinary air meter, so that the pressure is read in terms of cubic meters of flowing air per hour. If the manometer is read at the start and again at the finish of the run, the total amount of air that passed through the paper during the run can easily be calculated. At the end of the run the filter paper is removed from the collector and taken to the laboratory for analysis.

The paper with its collection of spores and pollen grains is treated with acetic anhydride and sulphuric acid, 9:1, heated to boiling on a water bath, to dissolve the filter paper and other unwanted material. The mixture is then centrifuged, the sediment washed to free it of the reagents, and suspended, in a measured volume of water. A sample of this is removed and placed in a specially devised counting chamber similar to that used for counting bacteria, but modified to adapt it to the much larger size of the pollen grains. The counting chamber enables one to learn the exact number of grains of each species of pollen per cubic centimeter of the aqueous pollen suspension. From this can readily be calculated the number of grains per cubic meter of air that passed through the Aerosol Collector during the run.

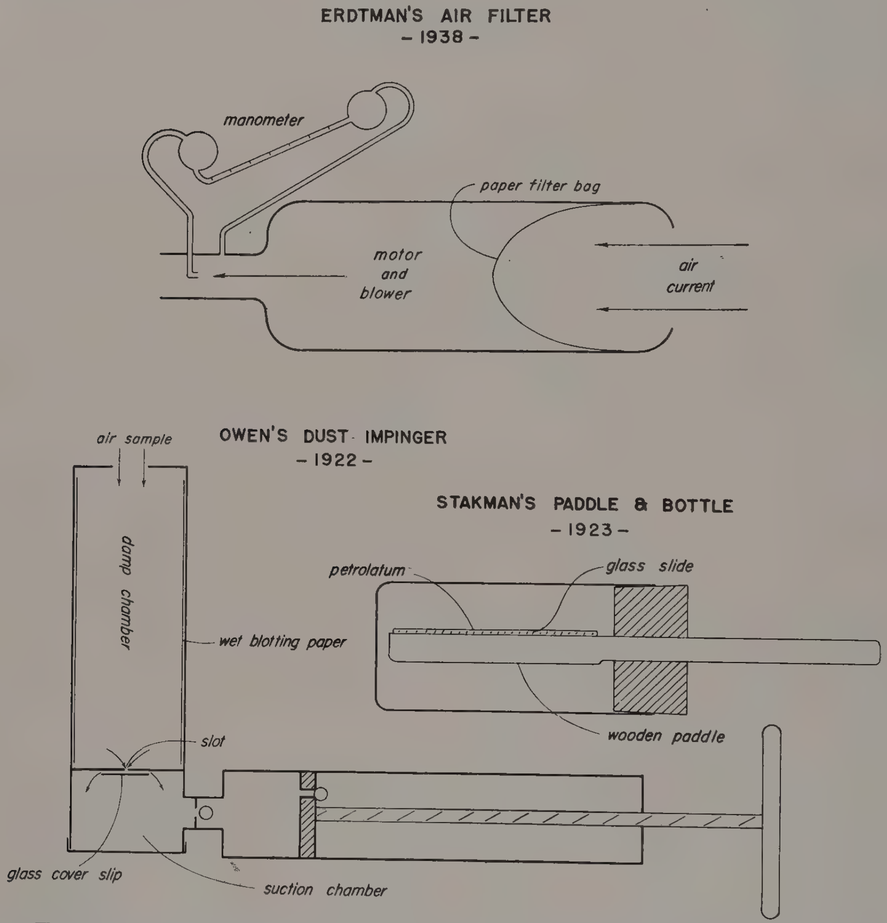

ERDTMAN'S AIR FILTER
- 1938 -
manometer
paper filter bag
motor and blower
air current
OWEN'S DUST IMPINGER
- 1922 -
air sample
damp chamber
wet blotting paper
slot
glass cover slip
suction chamber
STAKMAN'S PADDLE & BOTTLE
- 1923 -
petrolatum
glass slide
wooden paddle

FIG. 3. Above is Erdtman's air filter, used for determining the concentration of pollen in the air over the mid-Atlantic ocean. Below are Owens' and Stakman's devices used for catching dust particles and fungus spores during airplane flights.

Air Centrifuge

Wells (unpublished) has devised a type of rotating impinger especially adapted for pollen counting that impinges air onto a dry strip of celluloid (Fig. 2). The rotating parts of this apparatus consist of two concave face-to-face discs, spaced at their edges by the thickness of a cover glass and a strip of celluloid upon which the spores are precipitated. The arrangement provides a lens-shape chamber opening with a thin marginal slit through which air is projected onto a celluloid strip that rotates with the discs at high speed. The centrifuge revolves at about 4000 r.p.m., drawing air at the rate of about 3 cu. ft. per min. into the concavity between the two discs and precipitating the particles in the air onto the celluloid film in a narrow band suitable for microscope examination. Sampling the air consists simply of inserting a celluloid strip and spinning the instrument for about 10 minutes, which draws 1 cu. m. of air through the centrifuge. In making counts, more than 90 per cent of the microorganisms caught are found in 3 traverses along the strip with a lower-power objective. This device combines the principle of impinging with that of centrifugal precipitation.

MICROORGANISMS IN THE UPPER AIR

Pasteur and his colleagues pioneered in this work by opening sealed flasks of sterile media for short lengths of time at different altitudes on glaciated mountains.

Stakman *et al.* (81), Kimball and Hand (49), and Mischustin (59) were the first to use airplanes in this work. The former exposed 1 in. × 3 in. glass slides coated with vaseline and with glycerine jelly. Most of the exposures were made by means of slides fastened to paddles and kept in bottles before and after exposing (Fig. 3). When the plane reached the desired altitude the paddles were removed from the bottles and held out over the side of the cockpit.

Kimball and Hand (49) took samples by means of the Owens impinger (Fig. 3). This instrument is of special interest because it uses an entirely different principle from any other apparatus herein described, namely, the precipitation of dust particles in condensing moisture. The new feature of the apparatus is the damp chamber, which causes the air sample to become saturated with moisture. After saturation the air sample is drawn through a narrow slot at greatly reduced pressure, which causes the dust particles to be precipitated in droplets of dew on the glass cover slip placed opposite the slot. This apparatus has been widely used in dust-survey work.

Hubert (47), in 1932, described a spore trap designed by Schmidt that enables the operator to expose and change glass slides at a distance of 1 m. from the cockpit. His apparatus consisted of a long handle supporting a wooden cylinder with several glass slides attached and a box enclosing the cylinder, except for an opening arranged, so that one slide at a time could be exposed by rotating the cylinder.

Mischustin (59), Craigie and Popp (19), Brown (5), Meier, Stevenson, and Charles (56) and Walker (86) used glass slides and agar plates.

Proctor (66, 67, 68) brought air to his spore traps by means of a copper tubing that projected above the wing and was thus free from dust that might be blown off the plane. He passed samples of air through sterile fiber filters that were then disintegrated in sterile media and plated out in the usual manner. A venturi, placed on the plane wing, was used to draw the air through the filters arranged in a rotating magazine, as shown in figure 4. This equipment was modified later to permit aseptic microscopic examination and photomicrography of the collected particulate matter. The oiled slide impinger, based on the Pouchet apparatus, was incorporated and the culture methods simplified. Proctor and Parker have constructed recently an automatic sampling equipment based on the principles of this manually operated apparatus. In this equipment exposures are made on glass slides for definite time intervals, and provision is made for maintaining sterility and convenient subsequent analysis of the samples. An electrically controlled timing mechanism exposes successive slides to the incoming air stream and circulation is provided by a motor-blower in addition to that obtained by the positive pressure on the intake pipe, which is headed in the direction of flight. The intake system is kept closed by a protective cap prior to the time of sampling, is opened and held open magnetically, and closes when the last sample has been taken. The equipment is started by a switch on the instrument panel of the plane and operates automatically until 10 samples have been taken; then the equipment stops and is sealed without manual attention. Microscopic examination, photographing, and culturing are further simplified, and opportunities for contamination are practically eliminated. A photographic mechanism is incorporated to record the time of sampling, temperature, altitude, rate of air flow, and the number of samples being taken. This equipment has been calibrated in an aerodynamic wind tunnel and has been operated successfully in army airplanes. It is designed to fit directly into the aerial-camera mountings of army planes, but can be readily adapted to other types of aircraft.

Meier and Lindbergh (57) enclosed glass slides in separate metal cartridges, similar to Stakman's bottles, which were held in an apparatus that enabled them to remove the slide, expose it, and return it to the cartridge while the cartridge was at some distance from the airplane cockpit (Fig. 4).

The air scrubber of Zifferblatt and Seelaus (96) was adapted to airplane work by leading the air into the apparatus in much the same manner as Proctor did, except that a funnel-shape opening at the forward end of the tube forced the air through the apparatus rather than a venturi.

Mehta (55) used glass slides, attached to kites and balloons, to obtain an index of the number of cereal-rust spores in the upper air. Chatterjee (11), in 1931, gave a detailed account of the apparatus used with balloons, which apparatus included a trigger device activated by a fuse to time the ascent of the balloon and seal the slide after exposure.

Tsugi and Kurosawa (82) exposed vaseline-coated slides and Petri dishes in airplanes. Some of the exposures were made by means of a tube leading into the cabin from outside. More spores were caught on slides and dishes exposed at right angles to the air movement than on those held on an angle.

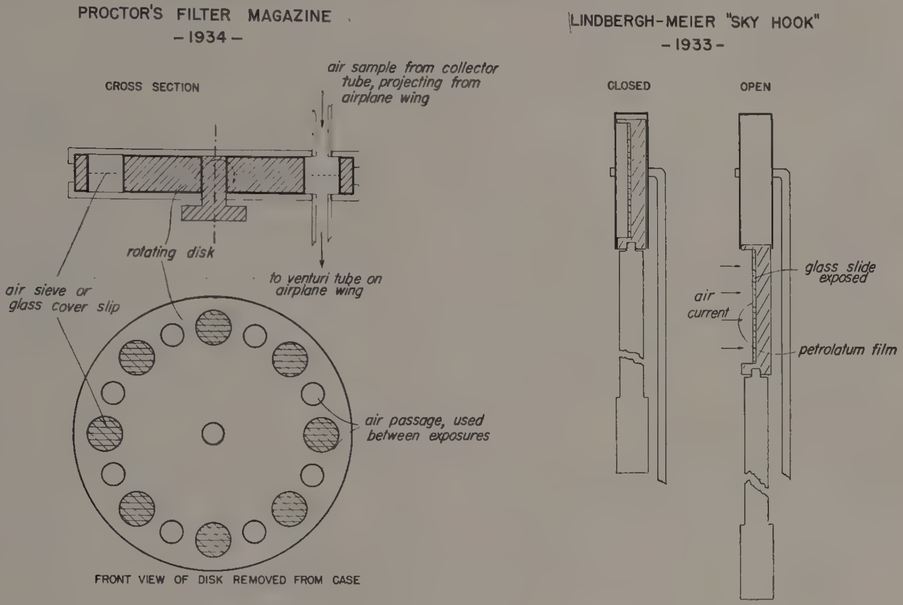

PROCTOR'S FILTER MAGAZINE
-1934-
CROSS SECTION
air sample from collector tube, projecting from airplane wing
rotating disk
to venturi tube on airplane wing
air sieve or glass cover slip
air passage, used between exposures
FRONT VIEW OF DISK REMOVED FROM CASE
LINDBERGH-MEIER "SKY HOOK"
-1933-
CLOSED
OPEN
glass slide exposed
air current
petrolatum film

FIG. 4. Apparatus devised by Proctor and by Lindbergh and Meier for catching spores during airplane flights.

EPIDEMIOLOGY OF PLANT DISEASES

Extensive studies of the dissemination of plant disease by means of air-borne spores have been made in relation to bunt of wheat, chestnut blight, white-pine blister rust, corn dry rot, apple scab, apple rust, and cereal rusts. Investigators in this field have used methods quite similar to those employed by the other groups, *i.e.*, gravity methods, filters, impinging aeroscopes, and airplane exposure of glass slides and Petri dishes. In addition, they have been able to trace the movement of spores in the air by observing the spread of infection in fields of susceptible plants.

In 1909 Burrill and Barrett (8) exposed funnels coated on the inside with adhesive in studying the movement of corn-rot spores. Heald, Gardener, and Studhalter (44) studied the dissemination of chestnut-blight spores in 1915 by several methods; glass slides, agar plates, Frankland sugar filters, and shallow dishes of sterile water. A few years later Heald and George (45) used shallow-dish water traps exposed to gravity precipitation of spores for studying showers of bunt spores occurring at harvest time. During the past 20 years, Stakman, Bailey, Craigie, and others have made extensive use of the method of exposing glass slides at the ground level in studying movement of cereal-rust spores.

Keitt and Jones (48) described a modification of Pasteur's gun-cotton filter in 1926, which they used for catching ascospores of the apple-scab fungus. Their apparatus consisted of a Pasteur filter unit, manometer, gas meter, and motor-driven suction pump. This enabled them to make accurate volumetric counts of the spores in air drawn through the filter at a rate of 1 cu. ft. per min.

Christoff (12) exposed slides in orchards by a modified Maddox aeroscope. The small end of the funnel on his aeroscope was about 1 cm. in diameter and the opening was spaced 3 cm. from the slide. Lactoglycerine was used as an adhesive.

The use of airplanes (81) and kites (55) in the study of epiphytotics of cereal rusts has already been described in the discussion of microorganisms in the upper air.

Spaulding (80) discusses studies on the wind dissemination of white-pine blister rust spores in which infections at various distances from known sources of inoculum were used as indices of distances to which spores were carried by the air. This method also has been used extensively by Stakman and others in the study of dissemination of stem rust of cereals from barberries to surrounding grain fields, and in tracing the movement of cereal-rust infection from south to north throughout the Mississippi Valley. Many analogous cases of studies of dust movement are cited by Free (36).

Buller (46), McCubbin (54), and Ukkelberg (84) have devised methods of studying the rate of fall of fungus spores in still air.

INSECT POPULATION AND MIGRATION IN THE AIR

For many years attention has been given to the insect population in the upper air, and references to observations and notes on insect migration and distribution through the upper air are numerous. Only in recent years, however, has any serious attention been given directly to methods of collecting data and material. Since 1926, a definite attempt has been made to collect and study insects in the upper air. Many collections have been made thousands of feet or even several miles above the earth's surface. Extensive studies also have been conducted on insect movements close to the earth's surface. The more important of these collections and investigations are herewith considered, with the respective methods, devices, and techniques used.

In general, the methods used have been adaptations of the time-honored net collectors and of adhesive-coated screens. Near the earth's surface large stationary screens have proved especially useful and motor-driven rotary traps appear to yield data approaching quantitative estimations of atmospheric insect populations. In the upper atmosphere airplanes and kites have been used extensively. Traps using attrahents are omitted from this discussion.

Rice (72), at the Idaho Agricultural Experiment Station, used special traps suspended from 6 inches to 65 feet above the ground. The traps were designed by Professor Claude Wakeland, and consisted essentially of a vertical screen panel, at the bottom of which on each side was attached a funnel leading through a small hole into a tin can in such a way that most insects, striking the screen and falling through the funnel, would be trapped in the can. It was possible to trap insects flying from two sides only. No attrahents of any kind were used, consequently only those insects that came into contact with the trap during their flights were captured. These traps were used on top of the Moscow Mountain Ridge, Idaho, to obtain information on the flight habits of the pea weevil, *Bruchus pisorum* (L.).

Riggert (73) studied the vertical distribution of fruit flies in Germany, using sticky screens. Screens were placed at intervals up to 60 feet in height.

Smith conducted experiments in 1930 and 1931 at Tallulah, Louisiana, to determine the near-the-surface population of the air using standard 3-by-5-foot sticky screens that were placed on a towerlike stairway with 3-foot intervals between each elevation of these screens, making the height of the screens above as follows: 3, 9, 15, 21, 27, 33, 39, 45, 51, and 57 feet, with the bottom of the first screen 3 feet above the ground, as in the field-flight screen studies.

Freeman (37) investigated the aerial distribution of insects by flying nets from the masts of the beam wireless station at Tetney, Lincolnshire, England.

Felt (32) made collections of insects from the roofs of tall buildings in Albany and New York City, etc.

Gaines and Ewing (38), during the period 1933–37, made collections of the flea hopper (*Psallus seriatus*) from traps. One trap at College Station, Texas, extended 26 feet into the air, and had 4 sections, each with a 5-ft. trapping surface, facing each of the cardinal directions. There was a space of 1 ft. between each 5-ft. section, for free circulation of air.

Fulton and Chamberlin used a wind-vane type of insect trap, which automatically received and retained the insects borne by a representative moving current of air in studies of insect dispersal and flight associations, with especial reference to the beet leaf hopper, *Eutettix tenellus* (Bak.). These traps were operated from spring to fall of 1930 in the Twin Falls-Jerome sugar-beet area of southern Idaho.

Chamberlin and Lawson (10) have recently described a motor-driven, mechanical, rotating trap (Fig. 5) for the sampling of aerial insect populations. The essential principal involved in the construction of this trap is that the power for its operation is so applied to one or more rigid insect nets as to cause them to rotate in a fixed horizontal plane at a spread sufficient to capture and retain in a removable collecting bag any insects that are intercepted. The utilization of this type of trap, with its adaptations, offers a quantitative method of determining the density of insect populations at different elevations and at different periods of the diurnal and annual cycles relative to such phenomena as time, temperature, humidity, and light intensity. Accurate records of insect populations can be obtained for the duration of periods as brief as 5 minutes or less or for as much longer as is desired. The trap has been employed principally, thus far, in studies of the flight habits and dispersal of the beet leaf hopper, *Eutettix tenellus,* and the pea weevil, *Bruchus pisorum* (L.).

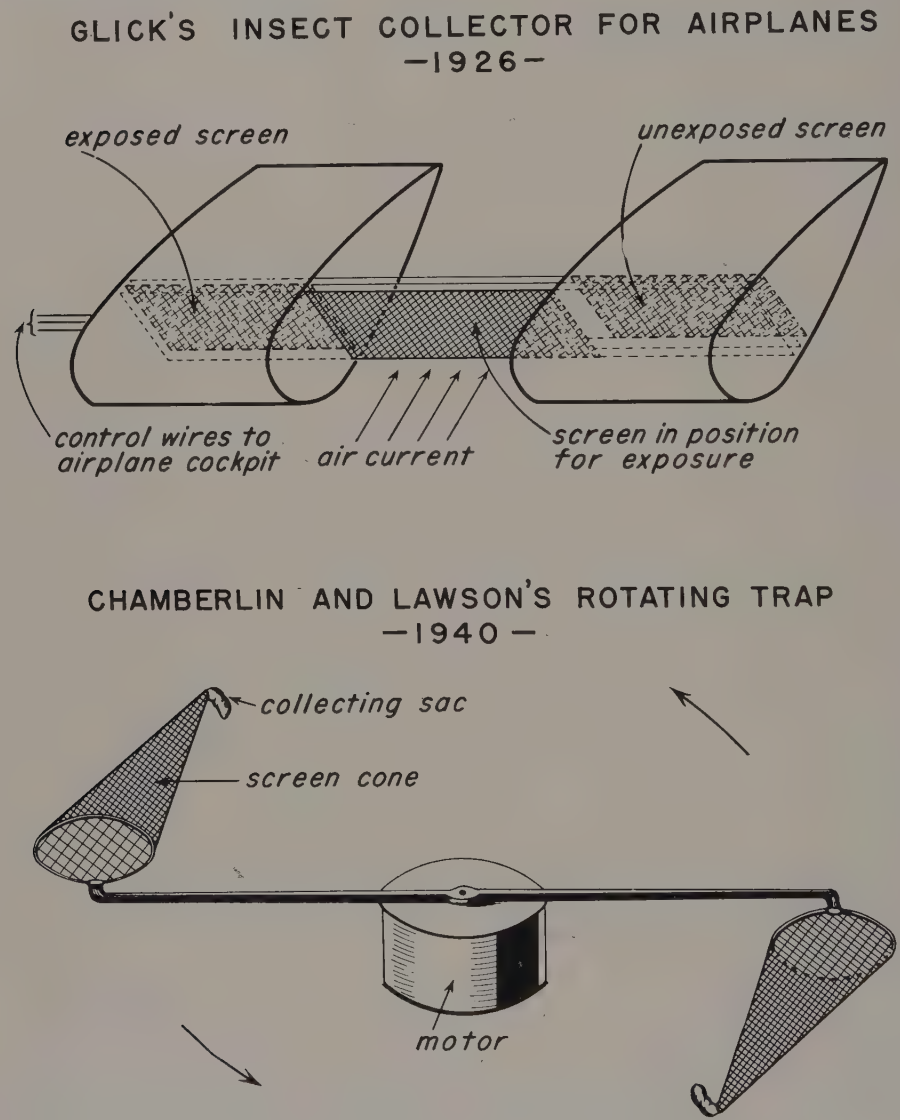

GLICK'S INSECT COLLECTOR FOR AIRPLANES
—1926—
exposed screen
unexposed screen
control wires to airplane cockpit
air current
screen in position for exposure
CHAMBERLIN AND LAWSON'S ROTATING TRAP
—1940—
collecting sac
screen cone
motor

FIG. 5. Equipment extensively used in insect distribution studies.

Glick (39) was the first to use an airplane for the direct purpose of collecting and studying the insect fauna of the upper air. The trap, as originally designed by him in 1926, consisted of 5 screen-covered frames for catching the insects, with suitable compartments, or magazines, for protecting the screens before and after exposure (Fig. 5). Refinements in construction, manipulations, and adaptation for use on different types of airplanes were made from time to time, but the basic principles remained unchanged throughout the work. Only one trap was used at first, then later two traps. They were placed either between the wings of a biplane or under the wing of a monoplane, one on each side of the fuselage or cabin. A single compartment trap was adapted for the use on the leading edge of the lower wing of a biplane, but the principle remained the same. At the desired altitude the screens were pulled one at a time into the open compartment and exposed for 10 minutes at a time. They were covered with a thin coat of adhesive to retain the insects that came into contact with them. Specimens were taken as high as 15,000 feet.

Gaines and Ewing (38), and Collins and Baker (17), have made extensive collections with traps, essentially as designed by Glick.

Felt (32) made airplane flights to collect insects in the upper air, using a trap with a sectional area of some 8 × 10 in., attached to the lower wings of a J. N. plane, and so arranged that glass slides, smeared with tanglefoot, could be exposed at given altitudes and for a definite time.

Berland (3), in 1934, was the first European to use an airplane to collect insects in the upper air. He used a net with a long cord fastened to the end of the wing of the plane. It was possible to keep the net in the cockpit of the plane until the desired altitude was reached, when it was let out gradually and flown suspended from the wing of the plane. The net was similar to the type used for plankton studies. The first half of the bag was made of a larger mesh to allow the air to pass through it freely, with a smaller and finer mesh for the second half of the net. The opening of the net was 12 inches in diameter, and about 28 inches in length, slightly conical and rounded at apex. The bag was attached to a hoop that kept the net rigid at the opening. A fine grade of medicated cotton was placed in the bottom or end of the net to allow the insects to collect on this as they were forced into the air stream of the net. This served also to keep the insects from being broken and crushed in contact. The net could be withdrawn into the cockpit of the plane during flight, and the cotton containing any specimens taken during exposure, removed and new cottom replaced to be used for another altitude. Berland made many flights over Paris and also in North Africa. Jeanne Larribère, accoucheure à l'Hôpital d'Oran, made a number of flights at Oran, Algeria (North Africa), using the Berland airplane insect nets.

Whitehead and Fenton used a net on a Cub airplane in collecting the green bug (*Toxoptera* sp.). A strut was conveniently located on the cabin in such a position that the lower edge of the insect net could easily be rested on the strut. With one edge on the strut and one hand holding the upper edge, the net could readily be held in position for a period of 5 minutes, which was the usual length of time the net was exposed. The smaller net could be readily held in position by simply holding the handle with the two hands approximately two feet apart on the end of the handle furthest from the net. A smaller net fitted within the larger net, and, after exposure, it was a very simple and easy task to draw the nets into the cabin of the plane, brush the insects into the tip of the small net, and secure them there by means of a rubber band, and place a new net into position for further collecting.

Parlato, LaDuca, and Durham (60) made observations on the emanations of the caddis fly (*Trichoptera*) by exposing glass slides on towers of buildings. The presence of these emanations could be definitely established by the examination of the exposed glass slides, the method being similar to that of pollen determinations.

Felt (32) used traps on various types of kites for the purpose of demonstrating possibilities of capturing insects in the upper air.

In 1933 the standard Weather Bureau kites were used in Montana to study the migration of the mountain pine beetle. Eight successful flights were made at elevations up to 8,000 feet.

Hardy and Milne (42, 43) studied the drift in insects over the North Sea in 1936. They used collecting nets flown either from the masthead or from kites flown from the ship.

They also made collections of insects in the upper air, using specially controlled kites. J. A. Freeman continued the work employing similar kites with additional improvements. The apparatus comprised a pilot kite of a triangular section box pattern with wide wings, having a span of 8 feet and a height of 8.5 feet. It was easily started in rather light winds, and flies very steadily at a good angle. A net resembling a marine tow-net was attached to the wire leading to the kite. The wire to the kite was controlled from the ground by a power winch attached to an automobile, and the kites having been launched could be veered out against the foot brake or hauled in by the engine working in reverse gear. When the closed net reached the desired altitude, it was automatically opened by a very simple, efficient, and light, chemically controlled mechanism, which worked by the action of nitric acid on copper wire. Later, Freeman constructed a clockwork mechanism for the control of opening and closing of the nets.

SUMMARY AND CONCLUSIONS

It is evident from the foregoing review that students of different phases of aerobiology employ a wide variety of techniques. In each field there are a few methods which seem to be especially well adapted for a particular kind of work. Conversely, each type of apparatus has its characteristic advantages and limitations.

For taking a census of bacteria and fungus spores that grow readily on culture media, the Wells' air centrifuge is outstanding in that it permits colony counts without further plating and it combines in one operation the development of an air flow, the measurement of the air flow, and precipitation of microscopic particles. The principal limitation of this method, as pointed out by Phelps and by Wells, lies in the fact that extremely small "droplet nuclei" may escape precipitation in comparatively large amounts.

The adhesive-coated glass slide has proved most convenient and is most widely used for determining the presence of pollen grains and fungus spores, such as rust spores, which can be recognized by direct examination but cannot be grown on culture media. Economy and simplicity of preparation and examination are the principal points in favor of the glass slide method. If slides are exposed to gravitational sedimentation of pollen or spores in comparatively still air for 24 hours, the number of pollen grains or spores caught on a square centimeter of slide surface can be easily converted to an approximation of the number per cubic yard of air by making use of Cocke's table. The chief limitation of this method appears to lie in the comparatively long exposure necessary to obtain satisfactory counts. Also, in the opinion of the writer, there is insufficient evidence for the tacit assumption that spore counts are not materially affected by currents of air immediately above the slide.

The glass-slide method becomes applicable to problems involving a more scant population of spores or pollen by moving the slide rapidly through the air, or by passing air rapidly over the surface of the slide. The former method has been widely used by Stakman, Meier, Durham, and others in making collections from airplanes. No attempt has been made to interpret counts made in this way on a quantitative volumetric basis.

Pouchet was the first to direct a current of air of known volume onto a limited area of a glass slide. Proctor and Parker have adapted this method to airplane collections, and find it quite satisfactory. The exposure of slides to a current of air caused by a small electric fan or blower, as proposed by Duke, would seem to offer a simple and inexpensive means of studying pollen or spores in concentrations smaller than would be noticed by the gravity method. All of these methods are essentially impinging methods, and their chief limitation lies in the difficulty of reliable calibration for the conversion of spore counts to quantitative approximations. They may be calibrated empirically by comparison with results from the filter methods, or centrifugal methods.

The Wells pollen centrifuge retains the simplicity of glass-slide methods in an apparatus that combines impinging with centrifugal sedimentation. It is claimed that this device completely removes from the air pollen grains and spores over 10 μ in diameter.

The air filter of Erdtman and Pasteur's filter method as modified by Keitt and Jones are well adapted for determining quantitatively the presence of spores and pollen that usually are detected by the glass-slide method. Erdtman's apparatus filters approximately 1 cu. m. of air per minute, whereas Keitt and Jones' filtered 1 cu. ft. per minute. The limitations of these methods lie in the necessity for dissolving the filters with a solvent that will not distort the spores or pollen grains, and the time consumed in preparing the filtered material for microscopic examination.

The air-washing methods employed by Rettger and by Zifferblatt and Seelaus eliminates the need for a filter solvent. On the other hand, these methods necessitate an examination of the filter liquid soon after passing air through it, whereas, solid filters may be stored after exposure until a convenient time for examination.

The field survey method used extensively by phytopathologists offers in the case of cereal rusts a unique opportunity to obtain evidence of spore migration, in that entire fields of susceptible grasses or grains are analogous to the Petri dishes exposed by the bacteriologist. Furthermore, an experienced observer oftentimes can determine the approximate dates of spore showers by the size and character of infection centers at the time of observation.

The methods used for catching insects are, as a rule, simply means of directing air flow through wire screens or nets and of protecting the screens from contamination before and after exposure. When the rate of air flow through the screens is known, it is comparatively simple to calculate from the number of insects caught the approximate number of insects present per unit volume of air.

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LITERATURE CITED

1. Acquarone, Paul, and L. N. Gay. A survey of the pollen flora in Baltimore during 1929. Jour. Allergy 2: 336–355. 1931.
2. Airy, Hubert. Microscopic examination of the air. Nature 9: 439–440. 1874.
3. Berland, L. Recherches en avio sur la faune de l'atmosphère. La Nature 2939: 341–345. 1934.
4. Blackley, C. H. Experimental researches on the cause and nature of Catarrhus aestivus. Lond. Med. Rec. 1: 373. 1873.
5. Brown, J. G. Living micro-organisms in the air of the arid southwest. Science 72: 322–323. 1930.
6. Buller, A. H. R. Rate of fall of fungus spores in the air. Nature 80: 186–187. 1909.
7. ————, and C. W. Lowe. Upon the number of microorganisms in the air at Winnipeg. Trans. Roy. Soc. Canada (3) 4: 41–58. 1911.
8. Burrill, T. J., and J. T. Barrett. Ear rots of corn. Ill. Agr. Exp. Stat. Bull. 133: 65–109. 1909.
9. Busse, G. Kiefernpollenflug und forstliche Saatgutanerkennung. Tharandt. Forstl. Jahrb. 77: 225–231. 1926.
10. Chamberlain, J. C., and F. R. Lawson. A mechanical trap for sampling of aerial insect populations. U. S. Dept. Agr. Leaflet (multigraphed). 1939.
11. Chatterjee, G. A note on an apparatus for catching spores from the upper air. Indian Jour. Agr. Sci. 1: 306–308. 1931.
12. Christoff, Alexander. Apparatus for catching the wind disseminated spore-balls. Zeitschr. d. Landwerkschaftlichen Versuchsstationen in Bulgarien 6: 41–48. 1934.
13. Clark, H. W., and S. DeM. Gage. A study of the hygienic condition of the air in textile mills with reference to the influence of artificial humidification. Mass. State Bd. of Health Ann. Rept. (1912): 661–692. 1913.
14. Cocke, E. C. Calculating pollen concentration of the air. Jour. Allergy 8: 601–606. 1937.
15. ————. A method for determining pollen concentration of the air. Jour. Allergy 9: 458–463. 1938.
16. Cohen, J. B. The air in towns. Smithsonian Misc. Coll. 39. 1896.
17. Collins, C. W., and W. L. Baker. Exploring the upper air for wind-borne gypsy moth larvae. Jour. Econ. Ent. 27: 320–327. 1934.
18. Cotter, R. U. Black stem rust spores combed from the air by fliers. Year Book, U. S. Dept. Agr. (1931): 116–118. 1931.
19. Craigie, J. H., and W. Popp. Rust epidemiology. Canadian Dept. of Agr. Exp. Farms Rept. of Dominion Botanist (1927): 47–54. 1928.

20. Cunningham, D. Douglas. Microscopic examination of the air. Calcutta, 1873. (Reviewed in Nature **9**: 330. 1874.)

21. Deamer, W. C., and H. E. McMinn. Studies in atmospheric pollen of San Francisco. Jour. Allergy **6**: 480–493. 1935.

22. Detwiler, H. K., and H. Hurst. Studies on the pollen content of the air. Jour. Allergy **1**: 334–345. 1930.

23. Duke, W. W. A rapid and more accurate method of determining the pollen content of the air. Jour. Am. Med. Assoc. **99**: 1686–1687. 1932.

24. ————. The dropping out of pollen seasons. Jour. Allergy **2**: 471–478. 1931.

25. ————. The pollen content of still air. Jour. Am. Med. Assoc. **91**: 1709–1711. 1928.

26. ————, and O. C. Durham. Pollen content of the air. Jour. Am. Med. Assoc. **90**: 1529–1532. 1928.

27. Durham, O. C. The contribution of air analysis to the study of allergy. Jour. Lab. and Clin. Med. **13**: 967–976. 1928.

28. ————. Cooperative studies in ragweed pollen incidence. Jour. Allergy **1**: 12–21. 1929.

29. ————. Incidence of ragweed pollen in the United States during 1929. Jour. Am. Med. Assoc. **94**: 1907–1911. 1930.

30. ————. The pollen content of the air in North America. Jour. Allergy **6**: 128–146. 1935.

31. Erdtman, G. Pollen grains recovered from the atmosphere over the Atlantic. Acta Horti Gotoburgensis, Meddelanden fran Götesborgs Botaniska Trädgard **12**: 185–196. 1938.

32. Felt, E. P. Dispersal of insects by air currents. N. Y. State Mus. Bull. **274**: 59–129. 1928.

33. Flügge, C. Die Verbreitungsweise und Bekämpfung der Tuberkulose. (Leipzig.) 1908.

34. Frankland, P. F. A new method for the quantitative estimation of the microorganisms in the atmosphere. Phil. Trans. Roy. Soc. (B) **178**: 113–152. 1887.

35. ————. Further experiments on the distribution of microorganisms in the air (by Hesse's method). Proc. Roy. Soc. **42**: 267–268. 1887.

36. Free, E. E. Movement of soil material by wind, with a bibliography of Eolian geology by S. C. Stuntz and E. E. Free. U. S. Dept. Agr. Bur. of Soils Bull. 68. 1911.

37. Freeman, J. A. Composition of the aerial insect fauna up to 300 feet. Nature **142**: 153–154. 1938.

38. Gaines, J. C., and K. P. Ewing. The relation of wind currents, as indicated by balloon drifts, to cotton flea hopper dispersal. Jour. Econ. Ent. **31** (6): 674–677. 1938.

39. Glick, P. A. The distribution of insects, spiders, and mites in the air. U. S. Dept. Agr. Tech. Bull. 673. 151 pp. 1939.

40. Guba, E. F., and F. M. Rackemann. Species of Cladosporium on tomato and the allergic response in man as an aid to their identification. Mycologia **30**: 625–634. 1938.

41. Hansel, F. K. Allergy of the nose and paranasal sinuses. C. V. Mosby Co. (St. Louis). 1936.

42. Hardy, A. C., and P. S. Milne. Insect drift over the North Sea. Nature **139**: 510–511. 1937.

43. ————. Studies in the distribution of insects by aerial currents. Experiments in aerial tow-netting from kites. Jour. Animal Ecol. **7**: 199–229. 1938.

44. Heald, F. D., M. W. Gardner, and R. A. Studhalter. Air and wind dissemination of ascospores of the chestnut-blight fungus. Jour. Agr. Res. [U. S.] **3**: 493–526. 1915.

45. ————, and D. C. George. The wind dissemination of the spores of bunt or stinking smut of wheat. Wash. Agr. Exp. Stat. Bull. 151. 1918.

46. Hogg, Jabez. The microscope. 1867.

47. Hubert, Kurt. Beobachtungen über die Verbreitung des Gelbrostes bei künstlichen Feldinfektionen. Fortschr. d. Landw. **7**: 195–198. 1932.

48. Keitt, G. W., and L. K. Jones. Studies of the epidemiology and control of apple scab. Wis. Res. Bull. 73. 1926.

49. Kimball, H. H., and Irving F. Hand. Investigation of the dust content of the atmosphere. Month. Weather Rev. **52**: 133–139. 1924.

50. La Rush, Florence. The pollen content of the air in Toronto, Canada, in 1932. Jour. Allergy **5**: 306–317. 1934.

51. Ludi, Werner, and Volkmar Vareschi. Die Verbreitung, das Blühen und der

Pollenniederschlag der Heufieberpflanzen im Hochtale von Davos. Ber. d. Geobot. Forschungsinst. Rübel in Zürich für das Jahr 1935. pp. 47–112. 1935.

52. Maddox, R. L. On the apparatus for collecting atmospheric particles. Month. Microscop. Jour. 3: 286–290. 1870.
53. Macdonald, J. D. Guide to the microscopic examination of drinking water. Appendix on the microscopical examination of air. Ed. 2, 1883.
54. McCubbin, W. A. Dispersal distance of urediniospores of *Cronartium ribicola* indicated by their rate of fall in still air. Phytopath. 8: 35–36. 1918.
55. Mehta, K. C. Rusts of wheat and barley in India. A study of their annual recurrence, life-histories and physiologic forms. Indian Jour. Agri. Sci. 3: 939–962. 1933.
56. Meier, F. C., J. A. Stevenson, and V. K. Charles. Spores in the upper air. (Abstract) Phytopath. 23: 23. 1933.
57. ————, and C. A. Lindbergh. Collecting microorganisms from the Arctic Atmosphere. Sci. Mont. 40: 5–20. 1935.
58. Miquel, M. P. Les organismes vivants de l'atmosphère. (Paris.) 1883.
59. Mischustin, E. Zur Untersuchung der Mikroflora der höheren Luftschichten. Centralbl. f. Bakt. etc. 67: 347–351. 1926.
60. Parlato, S. J., P. J. La Duca, and O. C. Durham. Studies of hypersensitiveness to the emanations of the caddis fly (*Trichoptera*). Ann. of Internat. Med. 7: 1420–1430. 1934.
61. Pasteur, L. Expériences relatives aux générations dites spontanées. Compt. Rend. Acad. Sci. 50: 303–307. 1860.
62. Penfound, W. T., and B. G. Efron. A standardized method for pollen air analysis. Proc. Soc. Exper. Biol. & Med. 27: 650–654. 1930.
63. Petri, R. J. Eine neue Methode Bacterien und Pilzsporen in der Luft nachzuweisen und zu zählen. Zeitschr. f. Hyg. 3: 1–143. 1888.
64. Pincus, S., and A. C. Stern. A study of air pollution in New York City. Amer. Jour. Pub. Health 27: 321–333. (Apr.) 1937.
65. Pouchet, F. Micrographie Atmosphérique-Moyen de rassembler dans un espace infinitement petit tous les corpuscules normalement invisibles contenus dans un volume d'air déterminé. Compt. Rend. 50: 748–750. 1860.
66. Proctor, B. E. The microbiology of the upper air I. Proc. Amer. Acad. Arts and Sci. 69: 315–340. 1934. (Contribution Dept. Biol. & Pub. Health, Mass. Inst. Tech. No. 29.)
67. ————. The Microbiology of the upper air II. Jour. Bact. 30: 363–375. 1935.
68. ————, and B. W. Parker. Microbiology of the upper air. III. An improved apparatus and technique for uppper air investigations. Jour. Bact. 36: 175–184. 1938.
69. Ransom, A. The atmospheric germ theory. Pop. Sci. Rev. 9: 209. 1870.
70. Rempe, H. Untersuchungen über die Verbreitung des Blütenstaubes durch die Luftströmungen. Planta, Arch. f. Wiss. Bot. 27: 93–147. 1937.
71. Rettger, L. F. A new and improved method of enumerating air bacteria. Jour. Med. Res. 22: 461–468. 1910.
72. Rice, P. L. Insects collected in flight traps in the vicinity of Moscow, Idaho. Jour. Econ. Ent. 26: 1079–1083. 1933.
73. Riggert, E. Ueber die Flughöhe der Fritfliege. Nachrichtenblatt Pflsch. Dienst. 11: 26–27. 1931.
74. Rowe, A. H. A study of the atmospheric pollen and botanic flora of the east shore of San Francisco Bay. Jour. Lab. Clin. Med. 13: 416–439. 1928.
75. Saito, K. Untersuchungen über die atmosphärischen Pilzkeime (I. Mittheilung). Jour. Sci. Coll. Imp. Univ. Tokyo, Japan 18: 1903–4.
76. ————. (II Mitteilung) Jour. Sci. Coll. Imp. Univ. Tokyo 23: 1908.
77. ————. (III Mitteilung) Japanese Jour. Bot. 1: 1–54, 1922.
78. Scheppegrell, Wm. Hayfever and hayfever pollens. Arch. Int. Med. 19: 959. 1917.
79. ————. Hayfever and asthma. Lea and Febiger (Philadelphia), 1922.
80. Spaulding, P. Investigations of the white-pine blister rust. U. S. Dept. Agr. Bull. 957. 1922. (Dissemination of sporidia, etc. See Bibliog. by McCubbin, York, York and Overholts, Pennington, Snell, etc.).
81. Stakman, E. C., A. W. Henry, G. C. Curran, and W. N. Christopher. Spores in the upper air. Jour. Agr. Res. [U. S.] 24: 599–606. 1923.
82. Tasugi, H., and E. Kurosawa. Fungi in the air. Ann. Phytopath. Soc. Japan 8: 35–42. 1938.
83. Tyndall, John. Spontaneous generation. Popular Sci. Month. 12: 591–604. 1877–78.

84. UKKELBERG, HARRY G. The rate of fall of spores in relation to the epidemiology of black stem rust. Bul. Torr. Bot. Club **60**: 211–228. 1933.
85. VAUGHAN, W. T. Allergy and applied immunology. C. V. Mosby Co. (St. Louis). 1934.
86. WALKER, G. Bacterial content of air at high altitudes. Sci. **82**: 442–443. 1935.
87. WELLS, W. F. Air-borne infection and sanitary air control. Jour. Indust. Hyg. **17**: 253–256. 1935.
88. ———, E. B. PHELPS, and C. E. A. WINSLOW. Ventilation and atmospheric pollution. IV. Report of sub-committee on bacteriological procedures in air analysis. A bacteriological method of sanitary air analysis. Yearbook 1936–37, Suppl. Amer. Jour. Pub. Health **27**: 97–101. 1937.
89. ———. Apparatus for the study of the bacterial behavior of air. Amer. Jour. Pub. Health **23**: 58–59. 1933.
90. ———, E. B. PHELPS, E. C. ROBERTSON, and C. E. A. WINSLOW. Report of sub-committee on bacteriological procedures in air analysis state of suspension. Yearbook 1939–40, Suppl. Amer. Jour. Pub. Health **30**: 99–104. 1940.
91. WILMER, H. B., and H. M. COBE. A note on quantitative survey of pollen. Jour. Allergy **3**: 63–68. 1931.
92. WINSLOW, C. E. A., JOHN WEINZIRL, J. B. THOMAS, M. J. ROSENAU, T. R. COWDEN, and GEO. A. SOPER. Progress Report of Committee on Standard Methods for the examination of air. Amer. Jour. Pub. Health **3**: 78–86. 1913.
93. WODEHOUSE, R. P. Atmospheric pollen. Jour. Allergy **4**: 220–226. 1933.
94. ———. Preparation of pollen for microscopic examination. Bull. Torr. Bot. Club **60**: 417–421. 1933.
95. ———. Pollen grains. The McGraw-Hill Book Co. (New York). 1935.
96. ZIFFERBLATT, A. H., and H. K. SEELAUS. A study of air contaminants in the interest of public health. Penn. Med. Jour. **39**: 67–73. 1935.
97. ZO BELL, CLAUD E., and HELEN M. MATHEWS. A quantitative study of the bacterial flora of sea and land breezes. Proc. Nat. Acad. Sci. **22**: 567–572. 1936.

PURPLE LEAF SPOT OF STRAWBERRY

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INTRODUCTION

In the fall of 1927, while attempting to isolate *Diplocarpon earliana* (Ell. and Ev.) Wolf, a slow-growing fungus was repeatedly isolated from strawberry leaves showing symptoms apparently typical of leaf scorch. This fungus was cultured on a variety of media, but failed to produce spores of any kind. A limited number of inoculations were made on strawberry plants, using a mycelial suspension of the fungus, but no infection was secured. In the meantime, the scorch organism (*D. earliana*) was isolated and the other fungus was discarded and forgotten. Then, 10 years later (in November, 1937), while again trying to isolate the leaf-scorch organism by plating strawberry-leaf-tissue pieces from what were thought to be typical young scorch spots, the same slow-growing fungus was repeatedly obtained, even when the leaf pieces were very drastically surface-sterilized with alcoholic mercuric chloride. This suggested the possibility that the fungus might not be a saprophyte but it might actually be the cause of the scorch-like spotting. Interest in the fungus was further aroused by the fact that one of the isolates produced in culture a limited number of perithecia bearing mature ascospores characteristic of the genus *Mycosphaerella*.

As a preliminary test to determine the pathogenicity of the fungus, 2 strawberry plants (var. Klondike) were inoculated, using macerated mycelium in water as inoculum. Infection was secured. The infected leaves were examined periodically from the time that the spots appeared until they were killed, but no conidia of any sort were found. However, when infected leaves that had begun to "scorch" were placed in a moist chamber, *Mycosphaerella* perithecia and ascospores developed.

It became apparent, therefore, from the preliminary work, (a) that the fungus so often isolated from leaves showing scorch-like lesions was a *Mycosphaerella,* and (b) that it was pathogenic and was, perhaps, the cause of a leaf spot with symptoms so similar to those of the scorch that it had been mistaken for it. Some interesting possibilities then presented themselves: (a) Was this fungus a strain of *M. fragariae* which had lost its ability to produce conidia in culture, or was it a new, undescribed species? (b) Since the published proof of the connection between *M. fragariae* and its conidial stage (*Ramularia tulasnei*) was meagre and inconclusive, it was considered possible that what had been described as *M. fragariae* might, in reality, be this fungus. An investigation of the fungus was, therefore, undertaken to determine its relation to the new leaf spot and its possible connection with *M. fragariae.* The results of this study have shown conclusively that the fungus in question has no connection with *Mycosphaerella fragariae* (Tul.) Lindau, but that it is a new, undescribed species of *Mycosphaerella* for which the name **M. louisianae** is proposed.

DESCRIPTION OF THE DISEASE

The macroscopic symptoms of the disease are, in general, very similar to those of the strawberry leaf scorch, and this may account for the fact that this disease has been previously overlooked. One might take Wolf's (14) description of the symptoms of the leaf scorch and apply it to this disease, and it would fit well with one important exception; there are no "dark, glistening acervuli" on the surface of the spots in the case of this disease. The spots are reddish purple when young, becoming brownish with age, but never develop grayish or white centers. Solitary spots vary in size from about 1 to 5 mm. in diameter (Fig. 1, A); but, when numerous, the spots coalesce, forming large, irregular, purple areas on the leaves (Fig. 1, B). In some instances, the large purple areas appear to be formed not by the enlarging and coalescing of separate spots, but rather by the simultaneous infection by large numbers of ascospores over a wide area. Sections through such purple areas, in their early stages of development, show a network of mycelium permeating the tissue (Fig. 3, A) and many remnants of germ tubes between the stomata. The spots are somewhat angular, and the edge of each is more prominent on the upper than on the under surface of the leaf. Compared with leaf-scorch spots of approximately the same stage of development, but not necessarily of the same age, because the scorch spots develop more rapidly, the spots are, on the whole, somewhat smaller, more angular, and less sharp in outline (Fig. 1, C and D). As in the case of the leaf scorch, "scorching" (drying up) of the affected leaves occurs if the spots are numerous and coalesced. Often the scorching may be limited to one leaflet, or part of a leaflet. Spots occur also on the petioles and fruit peduncles. The purple-spot disease often occurs on the same plant, even on the same leaf, with the common leaf spot (*Ramularia tulasnei*). No spores of any kind have been found on the lesions on living leaves. As soon as an affected leaf dies and begins to dry up, perithecia are produced in great abundance, mostly on the lower surface. The perithecia may occur in localized areas if the spots are few and scattered (Fig. 2, A), or may cover large irregular areas on the under surface of the leaf (Fig. 2, B) if the spots are numerous and coalescing. Viable perithecia have been found in all seasons of the year, but they have been especially abundant in late spring and early summer (April to June). It also is during this period of maximum ascospre production that the disease is most prevalent. During this period the leaves often become peppered with hundreds of small purple spots, obviously the result of ascospore infection, whence the fungus can be

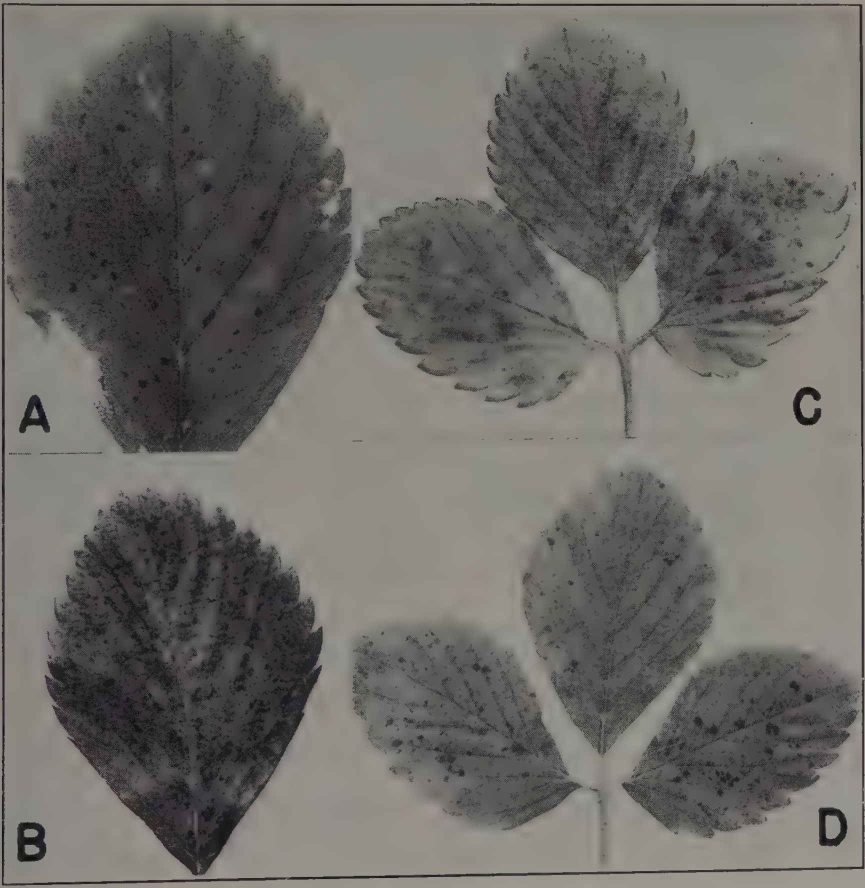

FIG. 1. Purple leaf spot symptoms. A. Individual spots. B. Very numerous spots, many coalescing to form irregular purple areas. C and D. Comparison between purple leaf spot (C) and leaf scorch (D) of approximately the same stage of development. × about $\frac{3}{4}$.

easily isolated. A second maximum outbreak of spotting occurs in the plant beds in the fall, when perithecia are observed in considerable abundance.

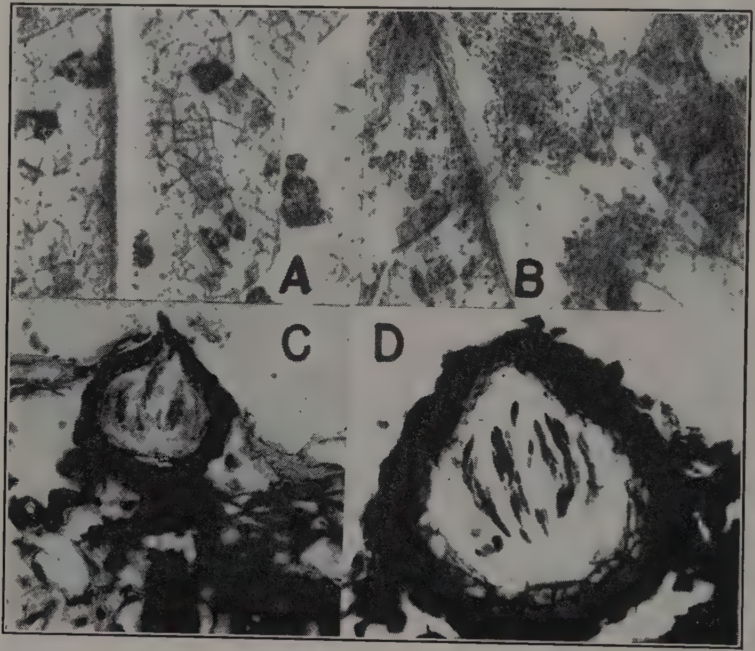

FIG. 2. A. Scattered groups of perithecia. B. Perithecia covering large irregular areas on under surface of leaf. ×6. C. Perithecium of *Mycosphaerella louisianae*. One ascus is protruding through the ostiolum. ×373. D. Perithecium of *M. fragariae* with mature asci. From overwintered leaves from Beltsville, Md., 1936. ×373.

GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

No special effort has been made to determine the geographic distribution of the disease. The fungus, however, has been isolated from material from North Carolina and Mississippi. The North Carolina material was collected by G. M. Darrow, near Whiteville, North Carolina, and was received on May 4, 1939. The Mississippi material was received from N. H. Loomis from Meridian, Mississippi, on June 6, 1939. Both collections consisted of perithecia-bearing dead leaves of the variety Klondike. In both cases the fungus was isolated by discharging ascospores on agar and making single-spore isolations. Infection was obtained from both the Mississippi and the North Carolina isolations. It is thus definitely known that, besides Louisiana, the disease occurs in Mississippi and North Carolina. Its incidence is quite probable also in other sections, especially in the South, where the Klondike variety is grown, since there has been considerable interchange of strawberry plants between different States. Because of its close resemblance to the leaf scorch, however, it has not been recognized as a separate disease. It is not limited to Klondike variety, but also has been observed on several of the seedlings in the strawberry breeding plots of the horticulture depart-

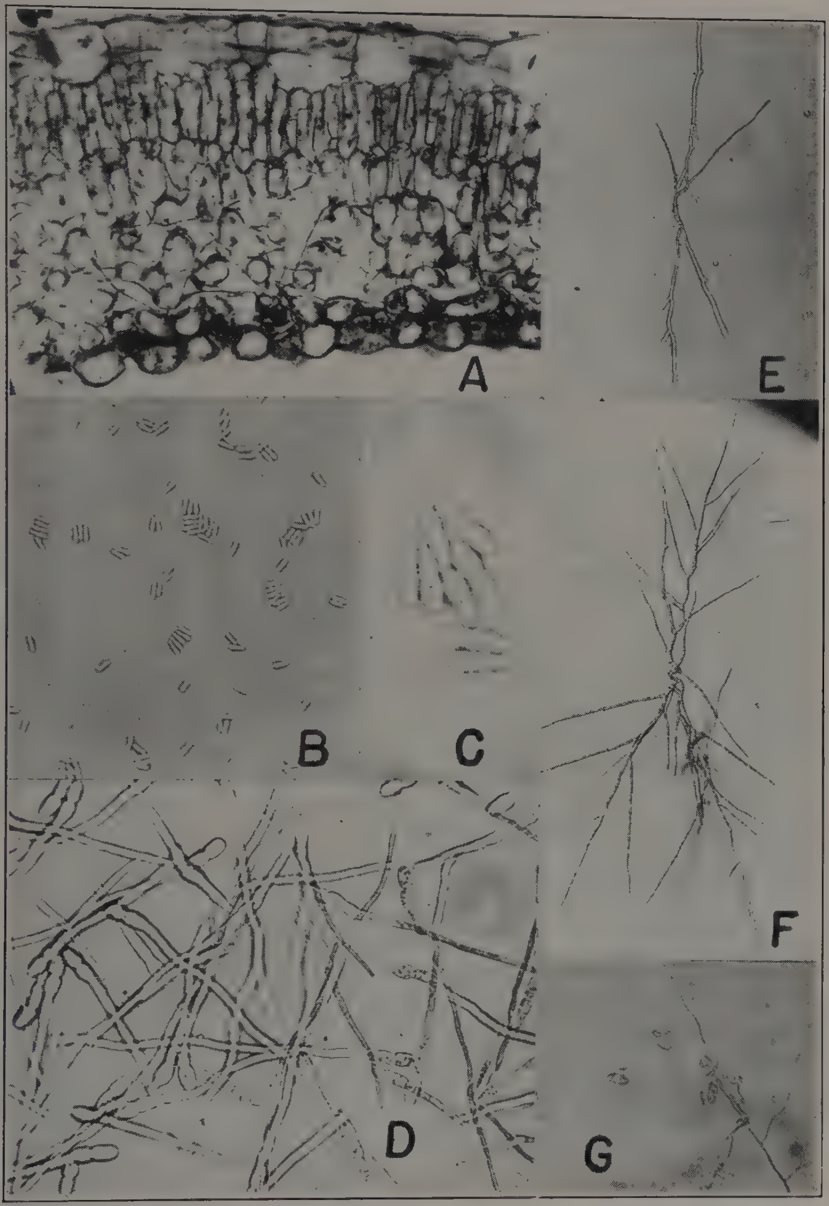

FIG. 3. A. Section through a leaf with irregular purple areas but with no definite spots showing a network of mycelium in the tissue. ×230. B. Ascospores from perithecia collected May 26, 1938, discharged on agar. ×230. C. Group of ascospores greatly enlarged. ×1010. D. Germinating ascospores about 25 hours after discharge on bean agar. ×370. E. Germinating ascospore 48 hours after discharge. ×125. F. Germinating ascospore 72 hours after discharge. ×125. G. Ascospores germinating on water agar and giving rise to secondary spores. ×230.

ment of Baton Rouge and at Hammond, and on the wild strawberry (*F. grayana*) in Washington Parish.

The disease is of relatively small economic importance, especially as its greatest incidence is in late spring when the crop is harvested. It has become clear, however, during the course of this study that the greater part of the leaf spotting we have been attributing to leaf scorch (*D. earliana*), in Louisiana, must be attributed to this new disease. The leaf scorch does occur in Louisiana, but is not particularly common. Most of the scorch-like spotting is caused by the new *Mysosphaerella.* The recognition that a separate disease is involved should not necessitate a change in our control measures, for spraying with Bordeaux, which has been the general practice in Louisiana during the last several years, has proved effective against all the leaf-spotting diseases of the strawberry.

THE FUNGUS

Isolation from Lesions at Different Periods of the Year

Strawberry leaves showing purple spots were collected periodically, and pieces of tissue containing young spots were plated. The following method, which has been found satisfactory in this laboratory for the isolation of other slow-growing fungi, was chiefly used. The spotted leaves were washed with a brush under running tap water, and pieces of tissue containing young spots were cut by means of a No. 1 cork borer. Then the pieces were disinfected either in a 3.5 per cent solution of calcium hypochlorite for 4 minutes and plated on bean agar without washing, or in 1:1000 $HgCl_2$ solution in 50 per cent alcohol for 30 seconds, and plated on bean agar after rinsing in several changes of sterile water. Both disinfectants gave satisfactory results. The plates were incubated at room temperature for 4 to 5 days, and then the tissue pieces showing no bacterial or fungous growth were transferred separately to autoclaved bean pods in tubes. The tubes were examined in about 10 days to determine what growth, if any, occurred on the leaf pieces.

The fungus was isolated from leaf lesions in November, 1937; in March, April, May, June, August, September, and November, 1938; and in January, April, and October, 1939. No effort was made to isolate the fungus in every month of the year, but it is evident that the disease occurs on strawberries in Louisiana in all seasons. Apparently, the fungus can be most readily isolated in May and June, and again in late fall. During these periods, perithecia are especially abundant on the dead leaves, and purple spots are very numerous on the living leaves.

Collection of Perithecia at Different Periods

Since no conidia were found either in culture or on the leaf lesions, it was at first puzzling to know how the fungus is perpetuated. It was thought, when the perithecia were found in the winter of 1938, that these would be produced only during the winter and early spring, as is usually the case with this group of fungi. A diligent search, therefore, was made for both conidia and ascospores. Conidia were never found either on naturally-infected plants in the field nor on inoculated plants, but viable ascospores were found at all seasons of the year. Viable perithecia on dead leaves were collected in January, February, March, May, June, August, September, October, and November, 1938. Perithecia also were found in abundance whenever looked for in 1939, but were relatively scarce in the winter of 1940. The viability of the ascospores was easily tested. The perithecia-bearing dried leaves were soaked in water about 5 minutes, washed with a brush in running tap water, and pressed between filter paper for a few minutes to absorb the excess moisture. Perithecia-bearing areas of the leaves were then cut and placed on moist filter paper in Petri-dish covers, and either suspended over agar or, by inverting the dish, the agar was on top to catch the ascospores as they were discharged upward. From living perithecia, ascospores were discharged in great profusion (Fig. 3, B) and almost 100 per cent germination occurred (Fig. 3, D) whenever ascospores were discharged.

The perithecia apparently die in a relatively short time on drying. Three lots of perithecia-bearing leaves were collected on May 16, 17, and 26, 1938. Samples of these were tested on the dates of collection, and ascospores were discharged in large numbers. The leaves were left in paper bags in the laboratory until June 28, 1938, when samples were taken and tested again. No ascospores were discharged. The leaves were then placed outdoors in a wire basket, and samples were taken and tested periodically until October, but the perithecia did not become active again.

Perithecia are produced just as soon as the infected leaf dies. Instances have been noted frequently in which one leaflet of a compound leaf, or even a portion of the same leaflet, would be killed, and perithecia would form on the dead portion, while the rest of the leaf would still be green. The time elapsing between infection and the production of perithecia was determined during the course of a number of inoculation experiments. In one test, six Klondike plants were inoculated with ascospores on September 29, 1938. First evidence of infection (pin-point spots) appeared 10 days later. By October 18 most of the inoculated leaves were covered with hundreds of coalescing purple spots, and "scorching" of portions of the leaves had started. Mature perithecia had developed on the dead portions of the leaves by November 4, *i.e.*, in 37 days after inoculation.

GROWTH OF THE FUNGUS IN CULTURE

The fungus was cultured on the following media: Bean-pod, Czapek's, and corn-meal agars, autoclaved bean pods, potato plugs, strawberry leaves, whole wheat, corn meal, and pea broth. It grew readily but slowly on all the media tried. The least growth was made on Czapek's agar, and the maximum on corn-meal agar; but, even on the latter medium, the colonies never attained any great size, the average diameter of three-week-old colonies on corn-meal agar being less than 15 mm. On corn meal and corn-meal agar, the growth was mostly flat, with a pinkish-white, felty surface and a pink to reddish back, the pigment diffusing into the substratum. On Czapek's agar, the colonies were small, raised, gray to nearly black, sclerotial, and more or less tough to rubbery in texture. On bean-pod agar the growth was raised, with a pinkish-white, felty surface that turned gray with age, and brown to dark-brown on reverse side. Perithecia with mature ascospores were often produced in culture, mostly on bean-pod agar; but a few were found also on autoclaved bean pods, strawberry leaves, and whole wheat, though none on corn meal or corn-meal agar. Only a very small number of the perithecia produced mature asci, and only a few of the asci in a perithecium produced mature ascospores. The production of ascospores in culture was very erratic. Sometimes they were produced very readily in about 10 days, and then again the same isolate would not sporulate for a long time. Some isolates sporulated more readily than others. The fungus is apparently homothallic, for many of the monoascospore isolates produced mature ascospores in culture. In addition to the perithecia, small groups of black sclerotial masses often occurred in cultures, especially on bean pods. These masses were found to be composed of 2 types of sclerotial growth, (a) small, spherical bodies, with or without cavities, which were probably immature perithecia, and (b) black sclerotial bodies of irregular shape, which were assumed to be true sclerotia.

No attempt was made to determine accurately the temperature range of the fungus. Only 3 temperatures were tried, namely, 21° C., 25° C., and 28° C. Best growth was obtained at 25° C. The size of colonies at 21° C. and 28° C. was approximately the same.

The germination of ascospores (Fig. 3, E–G) is very characteristic. Germination starts from the smaller cell first, and a second germ tube is produced from the other cell about 8 hours later. The germ tubes then begin to branch, the branches growing in opposite directions (Fig. 3, E), and, since growth is slow, the microscopic colony retains a double-fan or hour-glass shape (Fig. 3, F) for a relatively long time (3 to 4 days). The young colony becomes visible to the naked eye in about 5 to 6 days, by which time it has assumed a rounded form.

A very peculiar type of germination was observed on water agar (Fig. 3, G). The spores produced for the most part very short germ tubes (2 to 3 μ in length) at the end of which were formed secondary spores, shaped like the ascospores but smaller and unicellular. Occasionally, still smaller tertiary spores were produced from the secondary ones. Some of the germ tubes grew to a considerable length before forming secondary spores. One may speculate on the identity of these secondary spores. Should they be considered as secondary ascospores or as conidia?

PATHOGENICITY

The pathogenicity of the fungus was proved by repeated inoculations over a period of 19 months, from December, 1937, to June, 1939. Plants of the Klondike variety were used. These were potted from young runners and kept in an isolated place away from strawberry fields to prevent accidental infection, and used when needed. In all, 72 plants were inoculated. Two methods of inoculation were used.

1. Pure Culture Inoculum

Plants were inoculated with pure cultures of the fungus using a water suspension of macerated mycelium from bean agar or bean pods for the inoculum. This material was either sprayed on the leaves by means of a hand sprayer, or smeared on the leaf surfaces by means of a brush. The inoculum occasionally contained a small number of ascospores, if the culture used happened to be sporulating. Both monosporous isolates and isolates obtained from plating leaf lesions were used. In all, 42 plants were inoculated by this method.

2. Ascospores from Perithecia on Leaves

Plants were inoculated with ascospores obtained from perithecia on leaves in different ways. (a) Perithecia-bearing leaves were placed among the leaves of the plant to be inoculated, or suspended over them in a moist chamber. (b) Ascospore suspensions in water were obtained by suspending portions of perithecia-bearing leaves on moist filter paper inside the covers of Petri dishes and catching the discharged ascospores in water in the dishes. The ascospore suspension then was either sprayed or brushed on the leaves to be inoculated. (c) The same method as in (b), but the ascospores were caught on agar instead of in water. As soon as the spores began to germinate, bits of the agar containing ascospores were placed on marked areas on the upper or lower surface of the leaves. (d) Small portions of perithecia-bearing leaves were stuck on moist filter paper in small cells (van Tiegham cells or small bottle tops were used) and these were placed over marked areas on the upper or lower surfaces of the leaves, so that the ascospores would discharge directly on the desired leaf surface. The last two methods were applied mainly to determine whether infection takes place through the upper or the lower leaf surface, and also to determine the mode of ingress, *i.e.,* whether the fungus enters through the stomata or by penetration of the epidermis. In all, 30 plants were inoculated with ascospores.

In all experiments the inoculated and control plants were left in moist chambers for periods varying from 36 to 48 hours and then placed outdoors.

Results

In 8 out of the 72 experimental plants, inoculation was limited to the upper leaf surfaces. These 8 plants, as well as the many noninoculated controls, did not become infected. Of the remaining 64 plants, which either were purposely inoculated on the lower leaf surfaces only, or were inoculated by means of a hand sprayer, so that the inoculum reached both leaf surfaces, 63 became infected. Thus, the incidence of infection was very high. Typical symptoms, corresponding to those occurring in the field, were obtained consistently from the ascospore inoculations (Fig. 1, C). When a mycelial suspension was used as inoculum, symptoms that were not very typical were occasionally obtained. Sometimes the inoculated leaves assumed a brownish or russeted appearance, with few definite spots. Other times some of the spots would be surrounded with a chlorotic halo, or irregular areas on the leaf would become chlorotic before "scorching." Irrespective of the kind of inoculum and the symptom picture resulting from the inoculation, perithecia were, however, invariably produced on the inoculated leaves as soon as the tissues were killed. The latent period of infection (time elapsing between inoculation and appearance of external symptoms) varied considerably in different seasons of the year. In general, the first signs of infection (pin-point spots) appeared in 10 to 14 days, and definite spots in 19 to 21 days after inoculation. In the case of two separate inoculations made December 14 and January 11, respectively, the latent period of infection was very long, initial symptoms appearing in 24 days and definite symptoms in 37 days. Warm and moist conditions appear to favor infection and the expression of symptoms. The most severe infection was secured in a series of inoculations made in May, 1939. Initial signs of infection appeared in 10 days and good symptoms in 17 to 19 days. The weather was warm and very humid during this period.

MODE OF INGRESS

As stated in the preceding section, no infection was obtained when the inoculum was limited to the upper surface of the leaf. While it was apparent that infection occurred on the under surface of the leaf, it still remained to be determined whether the germ tubes entered through the stomata or by penetration of the epidermis. To determine this point, pieces of perithecia-bearing leaves were suspended on wet filter paper in small containers (bottle caps) and there were placed on marked areas on the dorsal side of leaves of plants in moist chambers and left there for 24 hours to discharge ascospores. The plants were then kept in moist chambers for 48 hours and then immediately taken outdoors. Pieces of the leaf areas exposed to ascospore discharge were taken at about 24-hour intervals, beginning 48 hours after inoculation, fixed, imbedded in paraffin, sectioned, and stained.

Results

As in the case of *Mycosphaerella fragariae* (8), ingress was found to be stomatal (Fig. 4, A–F). Sections from several different lots of material were examined, but entrance by penetration of the epidermis was never observed. Ingress is apparently slow. On material fixed 48 to 67 hours after inoculation, germinating ascospores with many germ tubes curving toward the stomata (Fig. 4, A, B) were found, but no actual ingress was noted. On material fixed 97 hours after inoculation, many germ tubes were found in the stomatal openings, but the tubes were still short, extending, for the most part, to the substomatal cavities only (Fig. 4, C) although some had begun to branch between the spongy parenchyma cells (Fig. 4, D, E). On material fixed 120 hours after inoculation, the mycelium had reached the palisade cells (Fig. 4, F) and there was branching considerably.

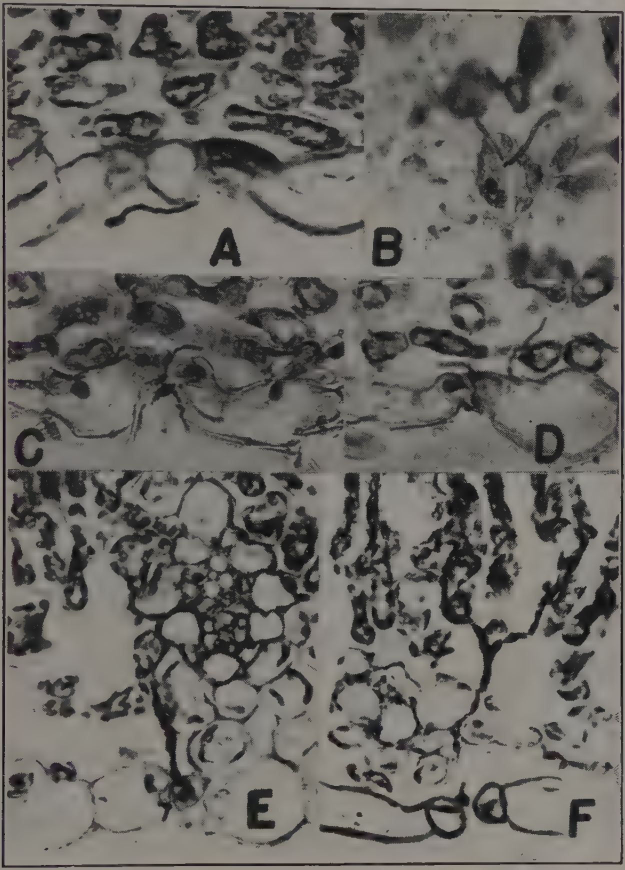

FIG. 4. Mode of ingress. A. Germinating ascospore, the germ tube curving toward stoma, 48 hours after inoculation. B. Surface section of leaf showing two germ tubes entering the same stoma, 67 hours after inoculation. C. Germinating ascospore, the germ tube of which has reached the substomatal cavity. D and E. Germ tubes have entered and are beginning to branch between the spongy parenchyma cells, 97 hours after inoculation. F. The germ tube has branched and has extended to the palisade cells, 120 hours after inoculation. All × 425.

DESCRIPTION

Perithecia are produced in abundance on the dead leaves in all seasons of the year, but are especially numerous in late spring (April to June). They occur grouped in black masses, easily visible to the naked eye (Fig. 2), mostly on the dorsal side of the leaves (over 90 per cent of the perithecia are hypophyllous). The number and size of the perithecial masses depend on the number, size, and degree of coalescing of the original leaf lesions. If only a few spots occur on the leaf, only few groups of perithecia will be found on the dead leaf (Fig. 2, A), but if the spots are numerous and coalescing, large areas on the dead leaf will be covered with groups of perithecia (Fig. 2, B). The perithecia are produced subepidermally, but become erumpent at maturity (Fig. 2, C). They are black, leathery, unilocular, and almost spherical, the short tapering ostioles making them slightly taller than they are wide. They vary considerably in size. Based on the measurement of 72 perithecia they varied from 34.0–75.0 × 34.0–75.0 μ, averaging 56.9 × 59.8 μ. The asci are fasciculate, short stipitate, hyaline, 8-spored, cylindric to clavate, those in the center of the fascicle straight, the marginal ones curved, 27.2–39.1 × 5.9–7.3 μ, averaging 32.7 × 6.4 μ in size. No paraphyses are present. The ascospores (Figs. 3, B–G, and 2, C) are imperfectly biseriate in the ascus, hyaline, ellipsoid, with blunt ends, 2-celled, the lower cell narrower than the upper, slightly constricted at the septum, 6.8–10.2 × 2.4–3.4 μ, averaging 8.9 × 3.1 μ in size, and contain many refractive granules.

A conidial stage has not been found either in culture or on the host. A diligent search for conidia was made by examining the lesions, both those occurring as the result of natural infection and those produced by inoculation, but none was found. Spotted leaves were often placed in moist chambers and examined periodically for conidia, but none developed. On the other hand, perithecia were invariably produced on the infected leaves, both on those placed in moist chamber and those attached to the plant, soon after they were killed. Since perithecia are produced in all seasons of the year, a conidial stage is not necessary for the perpetuation of the fungus.

Species of *Septoria* (probably *S. aciculosa* Ell. and Everh.) and *Cercospora* (9) were often isolated when tissue pieces from leaf lesions were plated, and the possibility that one of these might be the conidial stage of the new *Mycosphaerella* was considered. Both the *Septoria* and the *Cercospora,* however, produced conidia freely in culture, and their colonies did not resemble in the least those of the *Mycosphaerella.* Furthermore, the lesions produced by the *Cercospora* differ from those of the purple leaf spot, and no infection was obtained from inoculations with the *Septoria.*

No special attempt was made to determine if spermogonia are produced. In examining sections of material with perithecia, some fruiting bodies with filiform septate spores were often found, which resemble considerably those described and pictured by Longyear (7) as spermogonia of *M. fragariae.* In all probability these structures are pycnidia of *Septoria* rather than spermogonia of *Mycosphaerella.* Single-conidial isolations from similar structures on dead strawberry leaves yielded cultures of *Septoria.*

TECHNICAL DESCRIPTION

Mycosphaerella louisianae, n. sp.

Perithecia very numerous, in groups, amphigenous, but chiefly hypophyllous, partly imbedded in host tissue, erumpent, black, globose, ostiolate, 34.0–75.0 × 34.0–75.0 μ (average 56.9 × 59.8 μ); asci cylindrical to clavate, borne on short stalks, fasciculate, marginal ones curved, central ones straight, aparaphysate, 8-spore, 5.9–7.3 × 27.3–39.0 μ (average 6.4 × 32.7 μ); ascospores imperfectly biseriate in the ascus, hyaline, two-celled, the lower cell somewhat narrower, slightly constricted at the septum, obtuse, 6.8–10.2 × 2.4–3.4 μ (average 8.9 × 3.1 μ). Parasitic on leaves of *Fragaria* spp. (cultivated varieties) causing purple spots. The perithecia are produced in all seasons of the year soon after the infected leaves die.

A conidial stage is unknown.

Mycosphaerella louisianae, sp. nov.

Peritheciis numerosissimis, glomeratis, amphigenosis sed plerumque hypophyllosis, in hospitis textu ex parte insitis, erumpentibus, nigris, globosis, ostiolatis, 34.0–75.0 × 34.0–75.0 μ (mediis inter maxima minimaque 56.9 × 59.8 μ); ascis cylindratis vel subclavatis, e stirpibus brevibus prolatis, fasciculatis, exterioribus curvatis sed centralibus directis, aparaphysatis, octosporis, 5.9–7.3 × 27.2–39.0 μ (mediis inter m. mque 6.4 × 32.7 μ); sporis in ascis imperfecte biseriatis, hyalinis, cellas duas habentibus quarum superiore aliquantum maiore, ad septum leviter contractis, obtusis, 6.8–10.2 × 2.4–3.4 μ (mediis inter m. mque 8.9 × 3.1 μ). Parasitica in foliis *Fragariae* spp. (Varietatum cultarum) maculas purpureas efficiens.

Gradus conidifer incognitus est.

Perithecia omnibus anni temporibus pariuntur brevi spatio postquam folia infecta mortua sunt.

Type locality: Hammond, Louisiana, U. S. A.

Type specimens deposited in the herbarium of the Department of Botany, Louisiana State University, and also in the Mycological Collections, Bureau of Plant Industry, Washington, D. C.

IDENTITY

It is evident from the description of the fungus that it is a typical *Mycosphaerella* and cannot be confused with any of the other described leaf-spotting fungi of strawberry except *M. fragariae*. The possibility that it might be a nonconidial strain of *M. fragariae* has been considered. Cases of this kind have been demonstrated for other fungi. For example, Edgerton (3, 4), and Shear and Wood (12) have isolated strains of *Glomerella spp.* that produced only ascospores in culture. There is, however, strong evidence against the view that this fungus is an ascigeral strain of *M. fragariae*. First, the perithecia, asci, and ascospores of the new fungus are decidedly smaller than those of *M. fragariae* (Fig. 2, C, D). Scribner (11) gives the size of perithecia of *M. fragariae* as 90–130 μ with the asci about 50 × 10 μ; Klebahn (6) as about 90 μ; Schellenberg (10) as 100–150 μ; Duggar (2) states that the asci average 40 μ long and the ascospores about 15 × 3–4 μ. Flynn (5) gives the size of perithecia as 100–180 μ; the asci as 30–40 × 10–15 μ, and the ascospores as 12–15 × 3–4 μ. The writer examined herbarium specimens of *M. fragariae* collected by J. B. Demaree at Beltsville, Maryland, in 1936, and obtained the following measurements: Perithecia 82–115 × 88–136, average 99.3 × 113.0 μ; asci 30–45 × 10–15 μ; ascospores 12.6–15.3 × 3–4 μ, average 13.5 × 3.4 μ. Although there is considerable variation in the measurements of these workers, it is evident that the perithecia, asci, and ascospores of *M. fragariae* are markedly larger than those of the new fungus. Secondly, the positions of the perithecia of the two fungi differ. The perithecia of *M. fragariae* are predominantly epiphyllous (about 73 per cent were found on the upper leaf surface); those of *M. louisianae* are chiefly (over 90 per cent) hypophyllous. Thirdly, the symptoms on the host are different. The spots produced by *M. louisianae* are purplish and never develop the grayish-white centers characteristic of the *M. fragariae* lesions. Fourthly, none of the large number of isolates obtained either from single ascospores or from leaf lesions ever produced conidia. In all, 272 single-ascospore isolations were made from different lots of material and at different periods. These isolates were kept in culture over 2 years and examined periodically, but none produced conidia. It would be difficult to imagine such stability if it were assumed that this fungus is merely a nonconidial strain of *M. fragariae.*

There was another possibility that had to be considered, *i.e.,* that the fungus originally described as *Mycosphaerella fragariae* might be identical with the one described in this article as *M. louisianae.* If this happened to be true, the perfect stage of *Ramularia tulasnei* would still be unknown or, at least, not described. No reliable cultural proof of the connection between *Ramularia tulasnei* and *Mycosphaerella fragariae* was found in the literature.[1] Klebahn (6) states (p. 119): "Since Tulasne, the connection between *M. fragariae* and *Cylindrosporium,* which Saccardo later named *Ramularia tulasnei,* has often been discussed in literature. The assumption of the connection has been based entirely on Tulasne's finding of the two stages together. Up to now no one has, as far as I know, given proof of this connection."[2] Klebahn apparently overlooked Dudley's work. Dudley (1) suspended an entire ascus of *M. fragariae* in a hanging drop, and claimed that the ascospores germinated within the ascus, and gave rise to conidia of *Ramularia tulasnei* on the surface of the drop. Dudley's proof cannot be considered conclusive, since he used an entire ascus rather than single ascospores. Schellenberg (10) obtained infection, which resulted in typical leaf-spot lesions, by placing bits of overwintered leaf tissue bearing *Mycosphaerella* perithecia on the lower surfaces of strawberry leaves. He offers this as evidence of the connection between *M. fragariae* and *R. tulasnei.* This, at best, can be accepted only as circumstantial evidence, since it is known (11, 13) that conidia are produced on overwintered leaves from sclerotial mycelium and even from the walls of the perithecia. Wollenweber (15) accepted the connection between *R. tulasnei* and *M. fragariae* without experimental proof. Klebahn (6) attempted to get cultural proof, but was unsuccessful because few or no perithecia were produced on his experimental material. He states: "I have repeatedly overwintered leaves with Ramularia. Usually, only sclerotia were formed after overwintering. Several years ago I obtained one perithecium, and recently found two or three in material imbedded in paraffin and sectioned." He believed that the few perithecia found were stages of *R. tulasnei,* chiefly because they were similar to the perithecia of *M. hieracii,* which he had proved culturally to be connected with a similar conidial stage.

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[1] J. S. Flynn gives good cultural proof of the connection between *R. tulasnei* and *M. fragariae* in his unpublished Ph.D. thesis entitled "Mycosphaerella leaf spot of strawberry," 1928, which was deposited in the library of Cornell University. This thesis also contains an excellent review of the literature on the subject, especially on the nomenclature and classification of the causative fungus. The writer did not see this manuscript until February, 1939, when the present study was well under way.

[2] Free translation.

CULTURAL PROOF OF THE CONNECTION BETWEEN MYCOSPHAERELLA FRAGARIAE AND RAMULARIA TULASNEI

Since no reliable proof of the connection between *R. tulasnei* and *M. fragariae* could be found in the literature, it was decided to attempt to obtain such proof experimentally. Letters were written to a large number of plant pathologists in different states asking for over-wintered strawberry leaves which were known to have been affected with leaf spot the previous summer. The response was good. Specimens were received from Idaho, Montana, Minnesota, Wisconsin, Illinois, Michigan, Massachusetts, Pennsylvania, New York, and Maryland.

Results

The experience of the writer was, for the most part, similar to that of Klebahn. With one exception, all the specimens received contained mostly the winter sclerotial stage, although some had immature perithecia. No ascospores were discharged. The one exception was the material received from Michigan. This specimen consisted of over-wintered leaves of wild strawberry (*Fragaria virginiana*), collected by J. B. Routien near East Lansing, Michigan, on April 23, 1939. This material contained, in addition to the winter sclerotial satge, which was present in great abundance, a few mature perithecia. Of 18 plates prepared in the manner already described, using all the leaf material, ascospores were discharged in only two. In all, probably not more than 100 ascospores were discharged, and these fell, for the most part, close together, making isolation difficult. Five single-ascospore isolates were obtained. Several additional isolations were made in which the agar disc contained from 2 to 6 ascospores. All the isolations, regardless of whether they originated from single or multiple ascospores, gave rise to typical *Ramularia tulasnei* cultures and produced conidia in abundance. These cultures were morphologically indistinguishable from cultures of *R. tulasnei* from Louisiana obtained either from single conidia or from plating spotted leaf tissue. Thus, cultural proof of the connection between *M. fragariae* and *R. tulasnei* was obtained.

The scarcity, or lack, of mature perithecia, and the great abundance of the sclerotial stage on the overwintered leaves from widely scattered areas would indicate that the ascigeral stage of *R. tulasnei* plays a very minor role in the life history of the fungus, and is not essential to its overwintering, even in northern latitudes.

SUMMARY

A leaf spot of strawberry, with symptoms very similar to those of the leaf scorch, is described. The name "purple leaf spot" is proposed for it.

The causative organism of this hitherto undescribed disease was found to be an apparently undescribed species of *Mycosphaerella.* Evidence is presented showing that this fungus is distinct from *M. fragariae.* The fungus is described as a new species under the name *M. louisianae.* A conidial stage of the new fungus has not been found, either on the host or in culture. The fungus produces perithecia with mature ascospores in both winter and summer, and has produced a limited number of mature perithecia in culture. The new fungus was isolated from dead strawberry leaves sent in from Mississippi and North Carolina, so it is known to occur in at least two other States besides Louisiana.

Cultural proof of the connection between *Ramularia tulasnei* Sacc. and *Mycosphaerella fragariae* (Tul.) Lindau is also presented.

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LITERATURE CITED

1. DUDLEY, W. R. The strawberry leaf blight. Cornell Univ. Agr. Exp. Stat. Bull. 14. 1889.
2. DUGGAR, B. M. Fungous Diseases of Plants. Ginn & Co. (Boston). 263 pp. 1909.
3. EDGERTON, C. W. Plus and minus strains in the genus *Glomerella.* Amer. Jour. Bot. 1: 244–254. 1914.
4. ———. Effect of temperature on *Glomerella.* Phytopath. 5: 247–259. 1915.
5. FLYNN, J. E. Mycosphaerella leaf spot of strawberry. Unpublished Ph.D. thesis, deposited in the library of Cornell University, Ithaca, N. Y. 1928.
6. KLEBAHN, H. Haupt-und Nebenfruchtformen der Askomyzeten. XI + 395. Abb. 1–275. Gebrüder Borntraeger (Leipzig). 1918.
7. LONGYEAR, B. O. Fungous diseases of fruits in Michigan. Mich. Agr. Exp. Stat. Special Bull. 25: 55–56. 1904.
8. PLAKIDAS, A. G. The mode of infection with *Diplocarpon earliana* and *Mycosphaerella fragariae.* Phytopath. **24**: 620–634. 1934.
9. ———. Cercospora leaf spot of strawberry. (Abstract) Phytopath. **29**: 19. 1939.
10. SCHELLENBERG, H. C. Zur Kenntnis der Entwickelungsverhältnisse von *Mycosphaerella fragariae* (Tul.) Lindau. Vierteljahrschrift der Naturforsch. Gesell. (Zürich) **62**: 383–393. 1917.
11. SCRIBNER, F. L. Strawberry-leaf blight. *Sphaerella fragariae* Sacc. Report of the Commissioner of Agriculture (Rept. of the Chief of Sect. of Vegetable Pathology, pp. 334–341). 1887.
12. SHEAR, C. L., and ANNA K. WOOD. Studies of fungous parasites belonging to the genus *Glomerella.* Bur. Plant Ind. Bull. 252: 46–47. 1913.
13. TRELEASE, W. The spot disease of strawberry leaves (*Ramularia tulasnei* Sacc.). Wisconsin Agr. Exp. Stat. Ann. Rept. 2: 47–58. 1884.
14. WOLF, F. A. Leaf scorch disease of strawberries. N. C. Agr. Exp. Stat. Tech. Bull. 28. 1926.
15. WOLLENWEBER, H. W. *Ramularia, Mycosphaerella, Nectria, Calonectria:* Eine morphologisch-pathologische Studie zur Abgrentzung von Pilzgruppen mit cylindrischen und sichelförmigen Konidienformen. Phytopath. 3: 197–242. 1913.

LEAF–SPOT DISEASES OF POPLARS CAUSED BY SEPTORIA MUSIVA AND S. POPULICOLA[1]

G. E. THOMPSON[2]

(Accepted for publication September 10, 1940)

At least 9 species of the genus *Septoria* are reported associated with lesions on leaves of various species of poplar throughout the world. In certain regions of North America 2 of these, *Septoria musiva* Pk. and *S. populicola* Pk., are of common occurrence. In this paper the writer describes the perfect stages and certain other features of the life histories of these two fungi. The name *Mycosphaerella populorum,* sp. nov., is proposed for the perfect stage of *Septoria musiva* and *Mycosphaerella populicola,* sp. nov., as the name for *S. populicola.* In a recent paper Bier (1) has described a canker and leaf disease of poplars caused by *Septoria musiva.* As far as the writer has been able to determine, this paper is the only one in which information on the life history of this organism has been reported. None has been found relating to the life history of *S. populicola.*

MATERIALS AND METHODS

The species and varieties of poplars used experimentally were obtained from the Highland Park Arboretum, Rochester, New York; the Boyce Thompson Institute Arboretum, Yonkers, New York, and from the vicinity of Ithaca, New York. Inoculation experiments were performed on the following: *Populus acuminata* Rydb., *P. alba* L., *P. angulata* Ait., *P. balsamifera* L., *P. berolinensis* Dipp., *P. canadensis* var. *eugenei* Schelle, *P. canadensis* var. *serotina* Rehd., *P. candicans* Ait., *P. caudina* Tenore, *P. charkoviensis* Schr., *P. epirotica* Dode, *P. generosa* Henry, *P. incrassata* Dode, *P. kanjitaliana* Dode, *P. maximowiczii* Henry, *P. nigra* var. *gigantea, P. nigra* var. *italica* DuRoi, *P. nigra* var. *plantierensis* Schn., *P. nigra* var. *thevestina* Bean, *P. petrowskyana* Schn., *P. robusta* Schn., *P. roegneriana, P. simonii* var. *fastigiata* Schn., *P. szechuanica* Schn., *P. tacamahaca* Mill., *P. tremuloides* Michx. and *P. trichocarpa* Torr. and Gray. The cuttings obtained from these species were rooted in a mixture of loam and sand in 6-inch pots and kept in the greenhouse.

Inoculation experiments were conducted in the greenhouse. Conidia taken from freshly sporulating cultures were transferred to sterile distilled water in an atomizer. The suspension was sprayed on both surfaces of the leaves. After inoculation the plants were placed in a moist chamber for 48 hours. They were then transferred to greenhouse benches.

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[1] This paper constitutes a revised portion of a thesis presented to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer wishes to express his appreciation of the helpful criticism and suggestions of D. S. Welch under whose direction the work was done. He is indebted to H. M. Fitzpatrick for his aid in connection with the mycological aspects of the problem. Thanks are due also to H. S. Jackson, University of Toronto, D. H. Linder and G. D. Darker, Harvard University, H. D. House, Albany, N. Y., R. S. Horsey, Rochester, N. Y. and J. H. Beale, Yonkers, N. Y., for their kindness in sending specimens to the author. All photographs were taken by W. R. Fisher, Department of Plant Pathology, Cornell University.

For histological studies, small pieces of the desired leaf material were fixed in Flemming's weaker solution and embedded in paraffin. Microtome sections 5–7 μ thick were stained with Flemming's triple combination. Fresh material was studied from free-hand sections mounted in lactophenol cotton-blue combination.

PART I. LEAF SPOT CAUSED BY MYCOSPHAERELLA POPULORUM, SP. NOV.

The material used in the study of this leaf spot was collected in the vicinity of Ithaca. In this region the disease is common and occasionally destructive to the foliage of certain species of poplars.

SUSCEPTS

Inoculation experiments conducted by the writer demonstrated that the following members of the genus *Populus* are susceptible to this disease: *P. acuminata, P. angulata, P. balsamifera, P. berolinensis, P. canadensis* var. *eugenei, P. canadensis* var. *serotina, P. candicans, P. caudina, P. charkoviensis, P. generosa, P. incrassata, P. kanjitaliana, P. maximowiczii, P. nigra* var. *gigantea, P. nigra* var. *plantierensis, P. nigra* var. *thevestina, P. petrowskyana, P. robusta, P. roegneriana, P. simonii, P. simonii* var. *fastigiata, P. szechuanica, P. tacamahaca, P. tremuloides,* and *P. trichocarpa.* Two species, *P. alba* and *P. epirotica* remained free from any infection.

In literature other species reported as suscepts are: *Populus angustifolia* James, *P. deltoides* Marsh, *P. fremontii* Watson, *P. grandidentata* Michx., *P. occidentalis* (Rydb.) Britton, *P. rasumowskyana* Schn., *P. sargentii* Dode, and *P. monilifera* Ait. Since the nomenclature of the genus *Populus* is rather confusing, it is quite possible that certain of the names appearing above are synonyms.

THE DISEASE

Range

This leaf disease of poplars is known only in North America. It was described first by Peck (11) from specimens of *Populus monilifera* collected at Albany, New York. It has been reported since from Arkansas, California, Idaho, Missouri, Montana, New Jersey, Ohio, Texas, Vermont, and Wisconsin and has been observed by the writer in Georgia. In Canada, it is known to occur in the Provinces of Alberta, Manitoba, Ontario, Quebec, and Saskatchewan. As the fungus is capable of attacking many different species of *Populus,* it probably is present in other regions of North America.

Symptoms

The spots on the leaves of some species are small and numerous; on others they are large and relatively few. In general, they are circular, irregular, or angular, 1–15 mm. in diameter, reddish-brown to dark-brown, with narrow, yellowish borders (Fig. 1, B, C, D). The centers are frequently arid

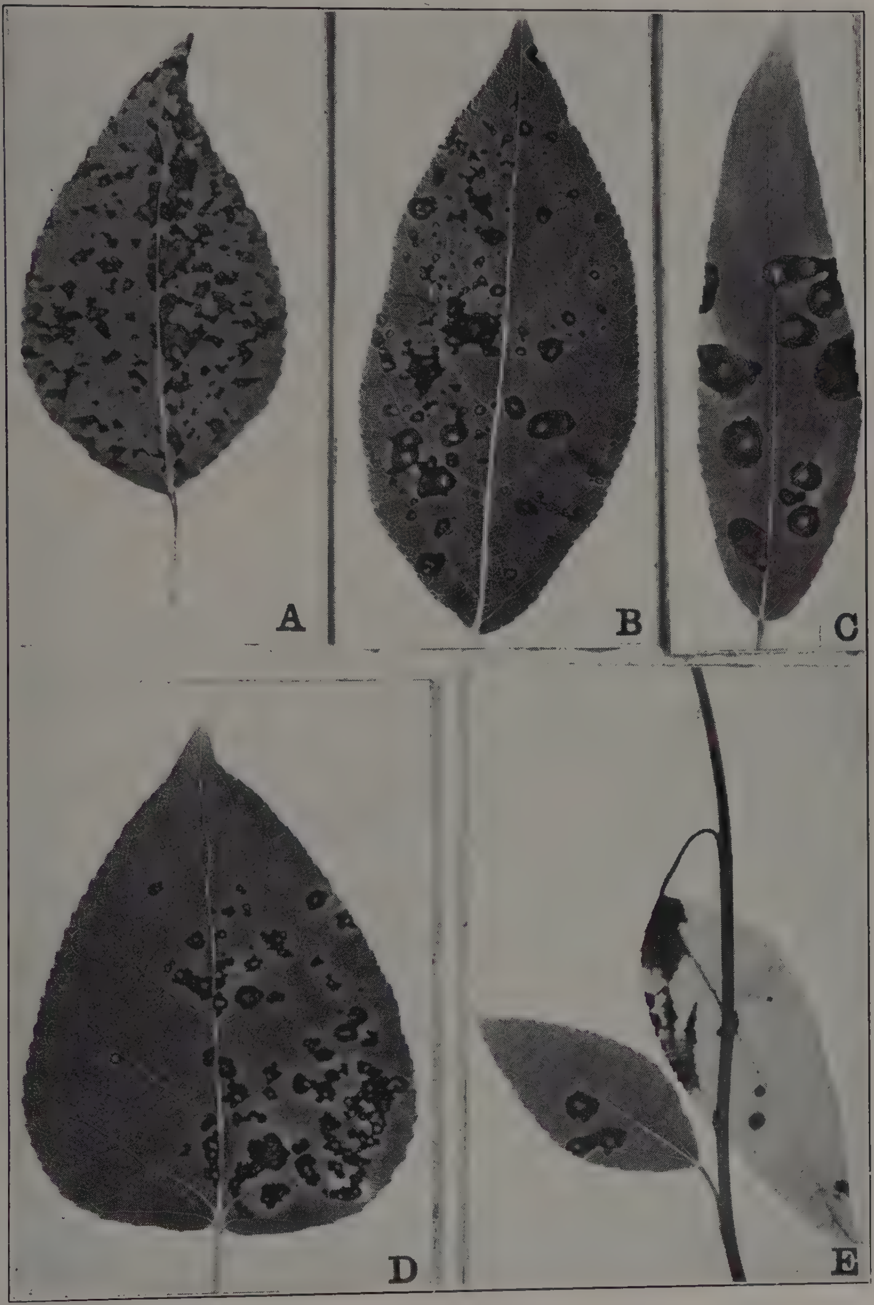

FIG. 1. A. *Mycosphaerella populicola.* Symptoms on *Populus tacamahaca.* B–E. *M. populorum.* B. Symptoms on *P. tacamahaca.* C. Symptoms on *P. szechuanica.* D. Symptoms on *P. candicans.* E. Twig canker on *P. szechuanica.* All slightly reduced.

or whitish. In some collections of infected leaves, the spots were distinctly zonate. The under surface of the lesions is usually lighter in color. Numerous infections resulted in yellowing throughout the leaf and premature defoliation.

Twig lesions or cankers were observed only on one shoot of *Populus szechuanica* (Fig. 1, E). Bier (1) has described this phase of the disease. He found that wound inoculations resulted in the formation of cankers on *P. berolinensis, P. petrowskyana, P. rasumowskyana,* and certain native hybrids.

Etiology

Perithecial Stage. Leaves of *Populus balsamifera,* bearing the conidial stage of *Septoria musiva,* were collected in the fall and wintered in wire baskets placed out-of-doors. Perithecia with the characters of the genus *Mycosphaerella* Johans. were found in these leaves in the spring. Three species of this genus on poplars are described from American specimens and the types of these were examined for comparison.

Mycosphaerella orbicularis (Pk.) House (7, 10) was described from wintered leaves of *Populus grandidentata* Michx. collected at Center, New York. Ascospores found in this material measured 11–14 × 2–3 μ. *Mycosphaerella populifolia* (Cke.) House (3, 7) was described from wintered leaves of *P. angulata* collected at Aiken, South Carolina. This species has groups of perithecia in erumpent, black stromata 0.25–1 mm. in diameter scattered over the under surface of the leaves. Free-hand sections of the stromata revealed locules with asci embedded in thick, hyaline pseudoparenchymatous tissue with dark, outer borders. Paraphysoids were interspersed between the asci. No mature ascospores were found in any of the material examined. The presence of a large amount of stromatic tissue around the locules and paraphysoids does not correspond to the genus *Mycosphaerella,* but indicates a closer relationship to the Dothideales. *Mycosphaerella populnea* (Sacc.) House (7, 14) was described from leaves of *P. balsamifera* (*P. tacamahaca*) collected at Tupper Lake, New York, August 22, 1913. Neither perithecia nor ascospores were found in the type specimens examined. Conidia typical of *Septoria populicola* were present in pycnidia in the leaf lesions.

The species of *Mycosphaerella* on poplars outside of North America are described with ascospore sizes that do not correspond to those of the fungus observed by the writer to be the perfect stage of *Septoria musiva.* Furthermore, since the conidial stage is known only in North America, the likelihood of the perfect stage having been described from outside regions is very remote.

Since there does not appear to be any species of the genus *Mycosphaerella* reported on poplars similar to the fungus under investigation, it is described as new and the following name is proposed:

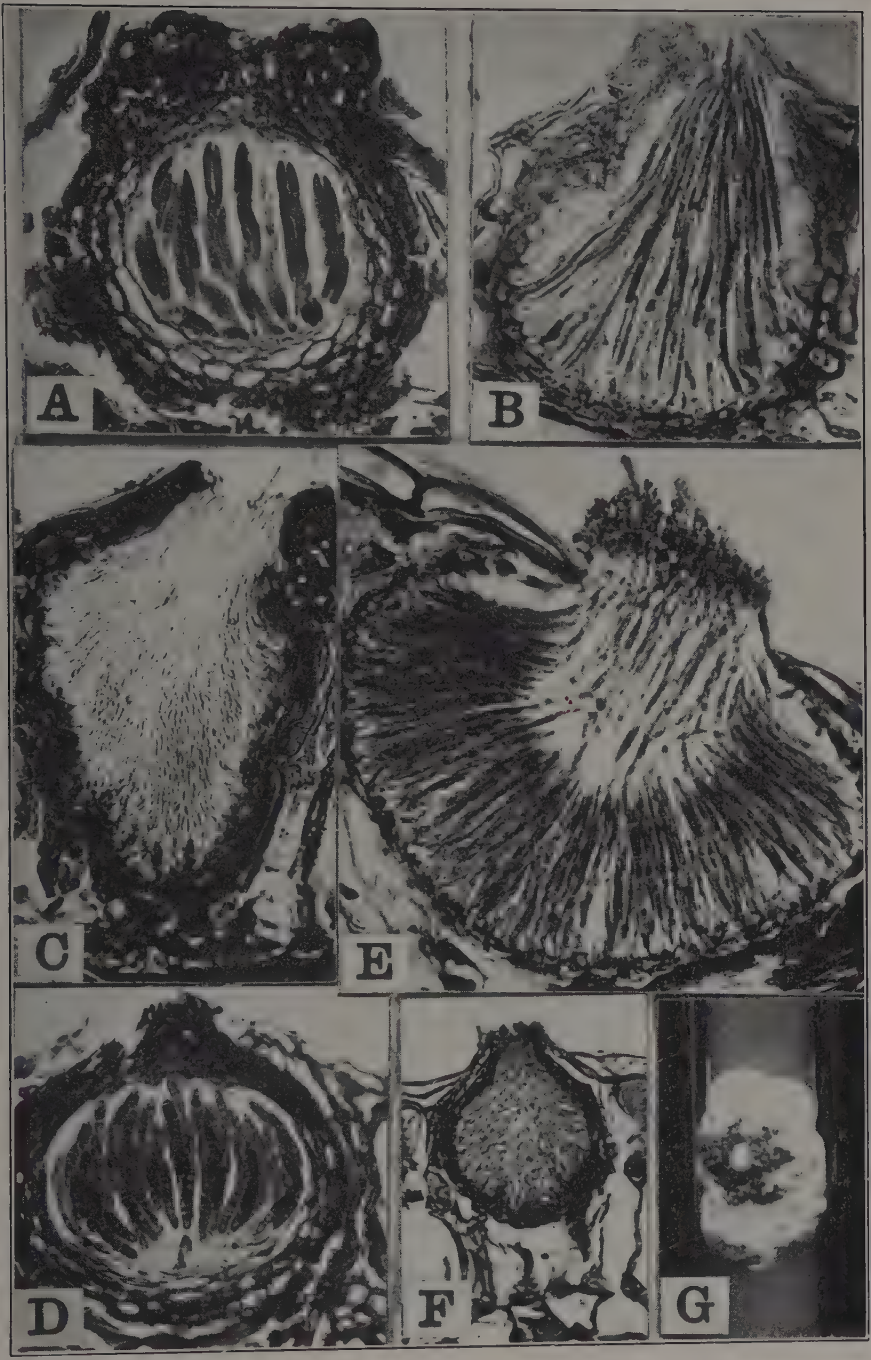

FIG. 2. A–C. *Mycosphaerella populicola*. A. Median section of a perithecium. ×430. B. Median section of a pycnidium. ×580. C. Median section of a spermogonium. ×600. D–F. *M. populorum*. D. Median section of a perithecium. ×430. E. Median section of a pycnidium. ×640. F. Median section of a spermogonium. ×430. G. Culture of *M. populicola*. Nat. size.

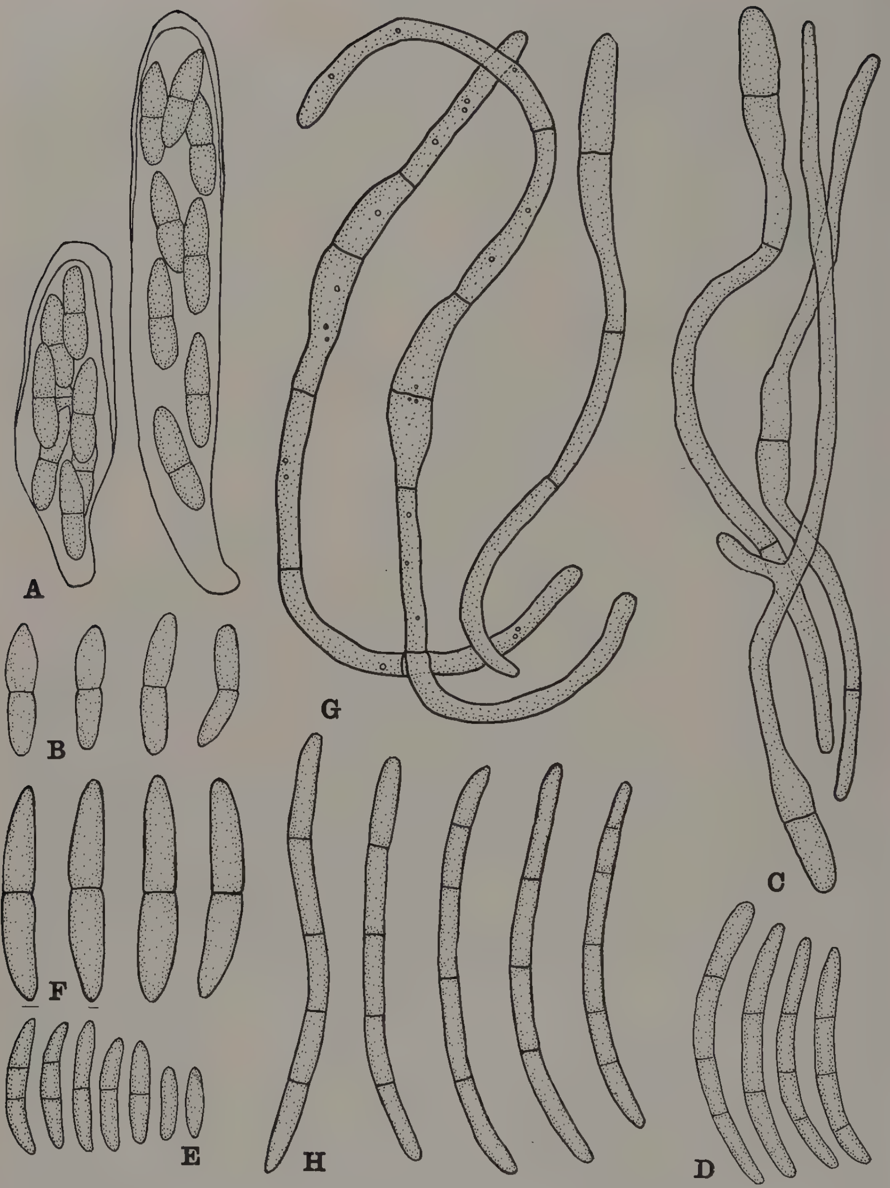

FIG. 3. A–E. *Mycosphaerella populorum.* A. Asci and ascospores. B. Ascospores. C. Ascospores germinated on potato-dextrose agar. D. Typical conidia. E. Atypical conidia. F–H. *M. populicola.* F. Ascospores. G. Ascospores germinated on potato-dextrose agar. H. Conidia. Drawings made with the aid of a camera lucida. × 900.

√**Mycosphaerella populorum**, sp. nov.

Syn. *Septoria musiva* Pk. Ann. Rept. N. Y. State Bot. **35**: 138. 1884.
Cylindrosporium oculatum E. and E. Jour. Myc. **5**: 155. 1889.

Peritheciis, amphigenis, sparsis vel aggregatis, semi-immersis, nigris, globosis, ostiolis papillato, 64–106 × 64–96 μ; ascis, fasciculatis, cylindraceis-clavatis, brevisme stipitatis, 54–70 × 13–16 μ, octosporis, aparaphysatis; sporidiis, irregulariter distichis, naviculatis, rectis vel curvatis, uniseptatis, leniter constrictis, cellulis plerumque aequalibus, hyalinis, 16–28 × 4.5–6 μ. Hab. in foliis dejectis vere *Populi balsamiferae* L.

Perithecia, amphigenous, scattered or aggregated, partially erumpent, black, globose, wall composed of 3–4 layers of dark, pseudoparenchymatous cells, ostiola papillate, 64–106 × 64–96 μ; asci, fasciculate, cylindrical-clavate, short stipitate, 54–70 × 13–16 μ, 8-spored, aparaphysate; ascospores, irregularly biseriate, naviculate, straight or slightly curved, one-septate, slightly constricted at the septa, cells about equal in size, hyaline, 16–28 × 4.5–6 μ (Fig. 2, D, and Fig. 3, A, B, C). Hab. in fallen leaves of *Populus balsamifera* L. in the spring.

Type Locality. Ithaca, New York.

Type Specimens. Author's collection No. 1325, part of which has been deposited in the following herbaria: Department of Plant Pathology, Cornell University, Ithaca, New York; Farlow Herbarium, Harvard University, Cambridge, Massachusetts; Department of Botany, University of Toronto, Toronto, Canada.

Pycnidial Stage. The characters of this stage of the pathogen are similar to those of *Septoria musiva* Pk. (11). Davis (5) regards *Cylindrosporium oculatum* E. and E. (6) as a synonym of *S. musiva.* The author confirmed this opinion after examining material of *Cylindrosporium oculatum.*

The pycnidia, which are produced throughout the summer, occur in lesions on both leaf surfaces, embedded in tissues below the epidermis. They vary from globose to depressed-globose. Walls consist of 1–2 layers of thin-wall, brownish cells. In some instances, walls very indistinct and delicate. Ostiola vary from narrow to wide, the latter giving pycnidia aspect of acervuli. Measurements of 112 pycnidia from 7 hosts showed them to have a range of 48–128 × 48–112 μ, average of 80 × 78 μ (Fig. 2, E). Average size of pycnidia, slightly greater on some hosts than on others, but the size ranges overlapped.

The conidia are cylindrical, sometimes tapering towards one end, straight or curved, hyaline and 1–4-septate (Fig. 3, D). Measurements of 850 conidia from 7 hosts showed range of 28–54 μ in length with an average of 37 μ. There was a slight difference in the average length of conidia from certain of the hosts but the ranges intergraded in all cases. The width of the conidia showed very little variation, being mostly 3.5–4 μ. Under moist conditions, the conidia oozed out from the pycnidia in pinkish masses or long spore tendrils, which became whitish upon drying.

Small white or arid spots sometimes were found intermingled with the typical lesions in field collections of infected leaves. Conidia obtained from pycnidia in these spots were elliptic-fusiform or cylindrical, straight or curved, hyaline, continuous or 1–2-septate, 12–29 × 3.5–4 μ (Fig. 3, E). The diversity in the dimensions of the pycnidia and conidia indicates that the fungus is a variable species as was pointed out by Davis (5) and Dearness (2).

Spermogonial Stage. As far as the writer was able to ascertain, this stage of the fungus has not been mentioned previously in literature. Spermogonia were found in lesions in the leaves on the trees and in fallen leaves on the ground in September and October. The globose spermogonia, 48–80 × 48–96 μ, occur scattered throughout the lesions on both leaf surfaces. They lie slightly sunken in the tissues below the epidermis, through which the narrow mouths protrude (Fig. 2, F). The walls of the spermogonia are composed of 1–2 layers of small, irregular, dark, cells. The spermatia, which ooze out at the mouths of the spermogonia in small glistening droplets, are unicellular, oblong or rod-shape, hyaline, 4–6.5 × 1.5 μ.

EXPERIMENTS ON THE DURATION OF ASCOSPORE DISCHARGE

Materials and Methods

Wire-screen wooden frames, 15 in. square, were used. Wintered leaves of *Populus balsamifera* bearing perithecia were spread out on the surface of one of the frames. The other frame was then placed over the leaves, so that they were held in position by the two screens. On April 29, 1936, the frames containing the leaf material were placed on a northeast hillside partly shaded by hardwoods and conifers. Clean glass slides were placed on the screens over the leaves. The slides were examined daily and fresh ones put in their place. Data on meteorological conditions were secured from the weather bureau located on the campus of Cornell University, about 300 yards from the frames.

Ascospores were first collected on May 1, and finally on August 29, 1936. In the interim, ascospores were found on the slides following rains of sufficient importance to moisten the leaves.

On August 29, 1936, wintered leaves containing perithecia of *Mycosphaerella populorum* were gathered from the ground beneath trees of *Populus balsamifera* and brought into the laboratory. The leaves were dampened and placed in a moist chamber lined with wet filter paper. Eight clean slides were placed over leaves. An hour later viable ascospores were found on each of 6 of the slides.

Since these experiments were conducted only in the summer of 1936, the results can be interpreted only as indicating that ascospore discharge may take place intermittently over a long period of time. The fact that the precipitation for May, June, July, and August, inclusive, of that year was 9.41 in. as compared with the normal of 13.88 in. may account in part for the length of the period of ascospore discharge.

PATHOGENICITY

In order to test the pathogenicity of the fungus, certain species of poplar, common to the vicinity of Ithaca, were inoculated under greenhouse conditions in 1933 and 1934. In 1936, additional species were inoculated. The results of all inoculations are presented in table 1.

Out of 28 species and varieties inoculated, 26 were susceptible and 2 remained free from infection. Pycnidia and conidia typical of the conidial stage of *Mycosphaerella populorum* developed in the lesions of all the susceptible species. Symptoms usually appeared 10–12 days after inoculation, although in some experiments they did not appear until after 20 days. Lesions usually appeared first on the older leaves. Young leaves, however, sometimes developed spots as soon as the more mature leaves.

PHYSIOLOGICAL STUDIES

The pathogen was isolated repeatedly in 1934, 1935, and 1936 from single ascospores, single conidia, and tissue plantings from infected leaves. The cultures obtained from these sources were similar to one another when grown on potato-dextrose agar, the only medium employed.

Characters of Growth in Culture

At the end of 3 days colonies of white, compact mycelium developed from single spores in cultures held at 24° C. After 10 days at this temperature,

TABLE 1.—*Results of inoculations with Mycosphaerella populorum under greenhouse conditions*

Tree species	Trees	Leaves inoculated	Leaves infected
	Number	*Number*	*Per cent*
P. acuminata	4	71	85.9
P. alba	5	93	0.0
P. angulata	1	38	28.9
P. balsamifera	7	126	46.0
P. berolinensis	4	80	27.5
P. canadensis var. *eugenei*	4	108	65.7
P. canadensis var. *serotina*	4	81	48.1
P. candicans	12	288	53.1
P. caudina	4	112	54.4
P. charkoviensis	2	40	35.0
P. epirotica	2	38	0.0
P. generosa	5	104	64.4
P. incrassata	4	91	56.0
P. kanjitaliana	4	150	15.3
P. maximowiczii	2	30	36.6
P. nigra var. *gigantea*	4	60	33.3
P. nigra var. *italica*	5	234	15.3
P. nigra var. *plantierensis*	3	58	24.1
P. nigra var. *thevestina*	2	45	64.4
P. petrowskyana	5	130	33.0
P. robusta	5	123	42.2
P. roegneriana	5	129	31.7
P. simonii	6	101	20.7
P. simonii var. *fastigiata*	5	185	31.8
P. szechuanica	6	169	65.6
P. tacamahaca	11	289	51.2
P. tremuloides	5	356	14.8
P. trichocarpa	7	145	66.8

the central portions of the colonies were greyish-green, surrounded by white borders. Growth took place in the centers in such a manner as to build these up. Small droplets of an amber colored liquid appeared upon the colonies. These droplets later became filled with conidia which oozed out into them in pinkish masses from pycnidia embedded in the mycelium. While the majority of the colonies had these characters, variations were observed. Certain colonies failed to form greenish centers and to produce conidia. Others showed intermediate characters in color and ability to sporulate. Isolations which were made from atypical conidia found in small white lesions formed colonies with greenish centers and white margins. In these cultures conidia were produced sparingly and did not develop until 10–12 days later than those that developed in cultures made from typical conidia.

Effect of Temperature on Germination of Ascospores

Freshly expelled ascospores were caught on cover slips, each bearing a drop of sterile water. Those showing a number of spores were inverted over van Tieghem cells and placed in constant-temperature chambers for germination. At the end of 6 hours, the germ tubes of 25 spores at each temperature were measured and averaged.

Under the above conditions, the optimum temperature for germination of ascospores was 27° C. At this temperature spores germinated in 75–90 min. with the formation of germ tubes at one or both ends of the spores (Fig. 3, C). At the end of 6 hours, the average length of germ tubes at this temperature was 76 μ. Germination took place at temperatures between 3° and 30° C. At 3° and 6° C. germination did not occur until 3–4 days had elapsed.

Effect of Temperature on Growth of the Fungus

Single ascospores were isolated and each planted in the middle of a test-tube slant of potato-dextrose agar. Two tubes were placed in each of a series of temperature chambers. At the end of 2 weeks the diameters of the colonies held at each temperature were measured and averaged. The maximum amount of growth occurred in the tubes kept at 27° C., where colonies attained an average diameter of 13 mm.

PART II. LEAF SPOT CAUSED BY MYCOSPHAERELLA POPULICOLA, SP. NOV.[3]

SUSCEPTS

Twenty-four species and varieties of poplar (Table 2) were inoculated under greenhouse conditions. Only *Populus szechuanica, P. tacamahaca,*

TABLE 2.—*Results of inoculations with Mycosphaerella populicola under greenhouse conditions*

Tree species	Trees	Leaves inoculated	Leaves infected
	Number	*Number*	*Per cent*
P. acuminata	2	52	0
P. alba	1	11	0
P. angulata	1	8	0
P. balsamifera	1	22	0
P. berolinensis	2	46	0
P. canadensis var. *eugenei*	2	43	0
P. canadensis var. *serotina*	4	101	0
P. candicans	4	106	0
P. caudina	1	44	0
P. generosa	2	40	0
P. incrassata	1	32	0
P. kanjitaliana	2	68	0
P. nigra var. *italica*	2	62	0
P. nigra var. *plantierensis*	5	146	0
P. nigra var. *thevestina*	2	38	0
P. petrowskyana	2	56	0
P. robusta	2	46	0
P. roegneriana	3	80	0
P. simonii	3	90	0
P. simonii var. *fastigiata*	2	74	0
P. szechuanica	3	120	50.0
P. tacamahaca	6	152	38.8
P. tremuloides	2	21	0
P. trichocarpa	4	104	48.0

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[3] The greater portion of the information reported in this section of the paper was obtained while the author was conducting field work in Northern Ontario for the Department of Botany, University of Toronto. The investigations were continued at Cornell University through the kindness of Professor H. S. Jackson who collected and sent fresh material to the writer.

and *P. trichocarpa* were susceptible; all others remained free from infection. In the literature *P. angustifolia, P. candicans* and *P. deltoides* are reported as hosts. The latter two were immune in the author's experiments. An examination of various exsiccati specimens showed further, that in certain collections the hosts were incorrectly determined and that *Septoria populicola* was confused with *S. musiva.*

THE DISEASE

Range

This leaf disease of poplars is known only in North America. In the United States, it has been reported from the following States: New York (12), Michigan (13), Vermont (8), Nebraska (9), and Idaho (15). In Canada it has been reported from Manitoba, Ontario, and Saskatchewan.

SYMPTOMS

The lesions on the leaves show considerable variation in size, shape and color and do not differ appreciably from those caused by *Mycosphaerella populorum.* Characteristically, they are 1–8 mm. in diameter, circular, and irregular or angular in outline. On the upper surface, the spots are reddish-brown to dark-brown, often with arid centers, and sometimes have a zonate appearance (Fig. 1, A). On the lower surface the spots are lighter brown. The spots may be sparsely or numerously scattered over the leaves. Frequently, coalescence of the lesions results in the formation of large spots occupying $\frac{1}{3}$ to $\frac{1}{2}$ the leaf area.

ETIOLOGY

Perithecial Stage. Leaves of *P. tacamahaca* bearing the conidial stage of the fungus, *Septoria populicola,* were collected in the fall and wintered in wire baskets placed out-of-doors. In the spring these leaves contained perithecia typical of the genus *Mycosphaerella.* Since a careful search through the literature and an examination of various exsiccati specimens of members of this genus on poplars showed that no species similar to the one under investigation has been described previously, it is here presented as a new species and the following name is proposed:

Mycosphaerella populicola, sp. nov.

Syn. *Septoria populicola* Pk. Ann. Rept. New York State Bot. **40**: 61. 1886.

Peritheciis, plerumque hypophyllis, sparsis vel aggregatis, semi-immersis, nigris, globosis, ostiolis papillato, 96–160 × 96–144 μ; ascis, fasciculatis, cylindraceis-clavatis, brevisme stipitatis, 64–90 × 13–16 μ, octosporis, aparaphysatis; sporidiis irregulariter distichis, fusiformibus, rectis vel leniter curvatis, uniseptatis, cellulis aequalibus, hyalinis, 22–32 × 6–6.5 μ. Hab. in foliis dejectis vere *Populi tacamahacae* Mill.

Perithecia mostly hypophyllous, scattered or aggregated, partially crumpent, black, globose, walls composed of 2–4 layers of rather large irregular, dark-colored cells, somewhat thickened around the ostiola, 96–160 × 96–144 μ; asci, cylindric-clavate, short stipitate, 64–90 × 13–16 μ, 8-spored, aparaphysate; ascospores irregularly biseriate, fusiform, straight or slightly curved, one-septate, slightly constricted at the septa, cells about equal in size, hyaline, 22–32 × 6–6.5 μ (Fig. 2, A, and Fig. 3, F, G). Hab. in fallen leaves of *Populus tacamahaca* Mill. in the spring.

Type Locality. Bear Island, Lake Temagami, Ontario, Canada.

Type Specimens. Author's collection No. 583, part of which has been deposited in the following herbaria: Department of Plant Pathology, Cornell University, Ithaca, N. Y.; Farlow Herbarium, Harvard University, Cambridge, Mass.; Department of Botany, University of Toronto, Toronto, Can.

Pycnidial Stage. The pycnidial stage of *Mycosphaerella populicola* was described as *Septoria populicola* by Peck (12) from material of living leaves of *P. balsamifera* L. (*P. tacamahaca* Mill.) collected at Keene, N. Y.

The pycnidia, which develop throughout the summer in the living infected leaves, are characterized as follows: pycnidia aggregated in the center of, or scattered irregularly throughout the lesions, amphigenous, globose or spherical, walls composed of 2–3 layers of dark-colored, irregular cells, walls occasionally thin and almost hyaline, ostiola protruding slightly, 80–167 × 80–167 μ (Fig. 2, B); conidia filiform or slightly clavate, straight or curved, 2–5-septate, hyaline, 45–80 × 3.5–4.5 μ (av. length 300, 62 μ) (Fig. 3, H), oozing out under moist conditions to form pinkish masses or tendrils, becoming whitish when dry.

Spermogonial Stage. This stage of the fungus apparently has not been mentioned in literature. Spermogonia were found associated with the pycnidial stage during August and September. A brief account of their morphology is as follows: Spermogonia amphigenous, globose or spherical, dark, sunken in the leaf tissues with the ostiola slightly protruding, walls composed of 2–3 layers of irregular, dark-color cells, 64–112 × 96–112 μ (Fig. 2, C) Spermatia, oblong or rod-shape, straight or slightly curved, unicellular, hyaline, 6.5–8 × 1–1.5 μ, oozing out at the ostiola in small glistening droplets.

PATHOGENICITY

During 1933 and 1934 inoculation experiments were carried out under greenhouse conditions, using species of poplars common in the vicinity of Ithaca, New York. In 1936, additional species of poplars were obtained for these experiments. The results of these experiments are presented in table 2.

The fungus was found to be pathogenic on three species of poplars. The remaining twenty-one species and varieties were free from infection. The symptoms usually appeared 14–20 days after the plants were inoculated. A longer incubation period was required when the summer temperatures in the greenhouse were high. One set of inoculations failed when the temperature in the greenhouse reached 46° C.

PHYSIOLOGICAL STUDIES

The fungus was isolated repeatedly during the course of three years from single ascospores, single conidia, and plantings of infected leaf tissue. Potato-dextrose agar was used as the culture medium.

Characters of Growth in Culture

Young colonies were white. With age, the centers became greenish and built up by mycelial growth. Pycnidia then developed partly embedded in the mycelium. Later these produced conidia which oozed out in pinkish masses (Fig. 2, G). The conidia produced in culture were slightly larger than those found in nature. Measurements of 100 conidia taken from cultures ranged from 54–99 × 4–5 μ with an average length of 76 μ. Spermogonia and spermatia that resembled those produced in nature were present in some of the older cultures.

Effect of Temperature on Growth of the Fungus

For these studies, single ascospores were isolated and planted singly in the middle of test tube slants of potato-dextrose agar. Two tubes were placed in each of 9 constant-temperature chambers. Two weeks later the diameters of the colonies grown at each temperature were measured and averaged. Those maintained at 21° and 24° C. showed the most abundant growth, with an average diameter of 12 mm. The fact that the optimum temperature for growth of the pathogen lies between 21 and 24° C. may account in part for the distribution of the disease, which occurs principally in the northern regions of the United States and in Canada.

SUMMARY

Part. 1. Leaf Spot Caused by Mycosphaerella populorum

In inoculation experiments conducted in the greenhouse, 26 species of poplar were susceptible to this disease. Two species remained free from infection.

The disease, known to occur only in North America, is widespread in the northern United States and in Canada.

The symptoms on the leaves are necrotic lesions, which vary in size, shape, and color.

The perfect stage of the fungus was obtained from wintered leaves of *Populus balsamifera*. It proved to be an undescribed species of *Mycosphaerella* to which the name *M. populorum* was given. In 1936, ascospores were discharged from perithecia, after rains, from May 1 to August 29. The conidial stage of the fungus was identified as *Septoria musiva* Pk. It developed in lesions on living leaves throughout the summer. Spermogonia containing minute, rod-shape spermatia developed in diseased leaves in September and October.

Isolations from ascospores and conidia usually gave the same type of growth. Variations between certain cultures were observed. The optimum temperature for germination of ascospores and for mycelial growth was approximately 27° C.

Part II. Leaf Spot Caused by Mycosphaerella populicola

Inoculation experiments conducted under greenhouse conditions on various species of poplar demonstrated that *Populus szechuanica, P. tacamahaca* and *P. trichocarpa* were susceptible to this disease. Twenty-one other inoculated species remained free from infection.

The symptoms are necrotic leaf spots that develop in summer.

The perfect stage of the fungus was obtained from wintered leaves of *P. tacamahaca,* which contained the conidial stage the previous summer. It is described as a new species and the name *M. populicola* is proposed. The conidial stage was identified as *Septoria populicola* Pk. It occurs in lesions on the living leaves in summer. Spermogonia, containing minute, rod-shape spermatia, were found in the lesions in the autumn.

Isolations from ascospores and conidia produced the same type of growth when grown on potato-dextrose agar. The optimum temperature for mycelial growth lay between 21° and 24° C.

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LITERATURE CITED

1. BIER, J. E. Septoria canker of introduced and native hybrid poplars. Can. Jour. Res. 17: 195–204. 1939.
2. BISBY, G. R., A. H. R. BULLER, and J. DEARNESS. The fungi of Manitoba. Longmans Green and Co. [London] 1929.
3. COOKE, M. C. On *Sphaerella* and its allies. Jour. Bot. 21: 107. 1883.
4. DAVIS, J. J. Notes on parasitic fungi of Wisconsin. Trans. Wis. Acad. Sci., Arts, and Letters 17: 870. 1914.
5. ———. Notes on parasitic fungi of Wisconsin. Trans. Wis. Acad. Sci., Arts, and Letters 18: 81–83. 1915.
6. ELLIS, J. B., and B. M. EVERHART. New and rare species of North American fungi. Jour. Myc. 5: 155. 1889.
7. HOUSE, H. D. An index to the New York species of *Mycosphaerella.* Ann. Rept. New York State Botanist 73: 25–31. 1920.
8. ORTON, W. A. A second partial list of the parasitic fungi of Vermont. Ann. Rept. Vt. Agr. Exp. Stat. 12: 175–176. 1899.
9. ———. Plant diseases in 1906. U. S. Dept. of Agriculture Yearbook [Washington] 1906: 507.
10. PECK, C. H. Ann. Rept. New York State Botanist 28: 81. 1879.
11. ———. Ann. Rept. New York State Botanist 35: 138. 1884.
12. ———. Ann. Rept. New York State Botanist 40: 61. 1886.
13. POLLOCK, J. B., and C. H. KAUFFMAN. Michigan fungi. Ann. Rept. Mich. Acad. Sci. 7: 60. 1898.
14. SACCARDO, P. A. Notae mycologicae. Ann. Myc. 13: 115. 1915.
15. SYDOW, H., and PETRAK, P. Ein Beitrag zur Kenntnis der Pilzflora Nord-amerikas, insbesondere der nordwestlichen Staaten. Ann. Myc. 20: 209. 1922.

A HERITABLE LYSIS IN GERMINATING CHLAMYDOSPORES OF SPHACELOTHECA SORGHI[1]

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(Accepted for publication Sept. 11, 1940)

INTRODUCTION

In the course of genetic studies on *Sphacelotheca sorghi* (Link) Clinton, the covered kernel smut of sorghum, it was observed that the chlamydospores produced by certain monosporidial crosses germinated in an abnormal manner and the promycelia disintegrated. A brief report of this phenomenon was presented before The American Phytopathological Society in 1938 (7). The present paper is a more detailed account and includes the results of further investigations.

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[1] Paper No. 1832 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

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LITERATURE

Abnormal germination of chlamydospores of several species of smut fungi has been reported in the literature from time to time. In *Ustilago zeae* (Beckm.) Ung., Christensen (2) found that the chlamydospores from a certain cross germinated abnormally, and sporidia, after budding several times, grew no further. Holton (5) reported that primary sporidia, isolated from the promycelia of germinating interspecific hybrid chlamydospores, produced by a cross between *Ustilago avenae* (Pers.) Jens. and *U. levis* (K. and S.) Mag., rarely developed in culture. He suggested that some lethal factor or factors may be present and become effective in certain interspecific crosses. Rodenhiser (8) found that promycelia from chlamydospores produced by an interspecific cross between *Sphacelotheca sorghi* Link (Clinton) and *Sphacelotheca cruenta* (Kühn) Potter were very abnormal and almost sterile. Goldschmidt (4) mentioned a "wild type" of germination occurring in chlamydospores of certain crosses of *Ustilago violaceae* Pers., which he attributed to cytoplasmic incompatibility. Kienholz and Heald (6) observed a rupture and degeneration and other abnormalities in the promycelia of germinating chlamydospores of *Tilletia tritici* (Bjerk.) Wint., which occurred on certain media only.

Recently, Chilton[3] (1) demonstrated that an abnormal type of chlamydospore germination in *Ustilago zeae* was governed by a genetic factor or factors that caused the promycelia to disintegrate or "lyse." The lysis appeared in all crosses in which the lethal factor or factors involved were present in one of the parental lines and absent in the other. The abnormality persisted through 3 chlamydospore generations. Chilton observed also that solopathogenic lines were obtained relatively often from crosses involving the lethal factor or factors, which suggested that abnormal meiotic divisions may have occurred.

The lysis observed in *Sphacelotheca sorghi* resembles that observed by Chilton in *Ustilago zeae*, but differs from it in certain respects, especially the lack of ability to produce solopathogenic lines and the effect of the lethal factors on the morphology of germinating chlamydospores.

ABNORMAL GERMINATION IN SPHACELOTHECA SORGHI

Cross 22A3 × 122B3. Abnormal germination was first noticed in the chlamydospores resulting from crossing two monosporidial lines, 22A3 × 122B3. In this cross the chlamydospores germinated and formed promycelia normally, but no sporidia were produced within 48 hours, and the

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[3] Chilton, S. J. P. Further studies on the cytology and genetics of *Ustilago zeae* (Beckm.) Ung. Unpublished Ph.D. thesis, University of Minnesota. 1938.

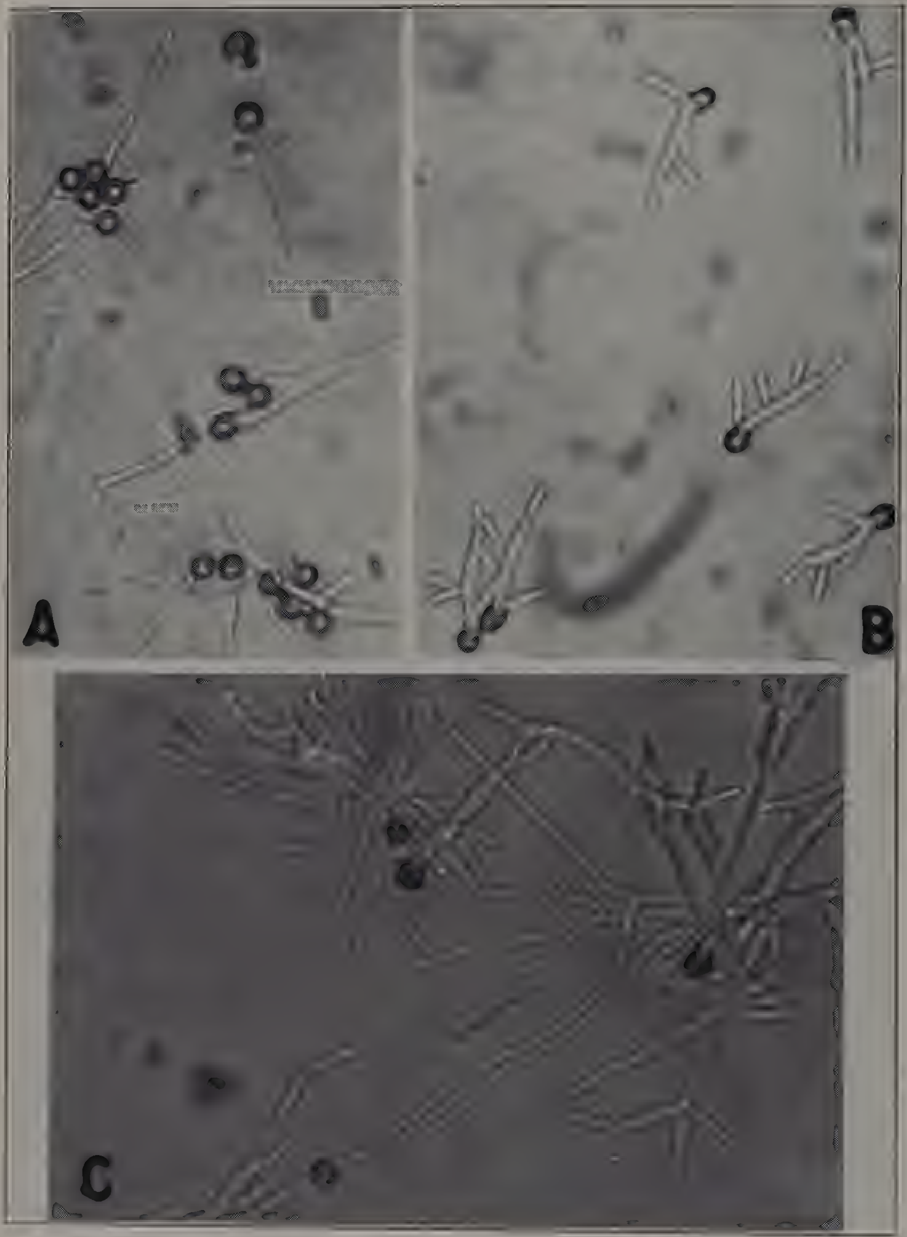

FIG. 1. Lysed promycelia of chlamydospores produced by a lethal cross $(22A_3 \times 122B_1)$. B. Typical germination of chlamydospores produced by a non-lethal cross. C. Abnormally germinating chlamydospores of cross $22A_3 \times 122B_3$. Some promycelia are producing almost normal sporidia while others are producing atypical sporidia.

promycelia finally disintegrated (Fig. 1, A). Normally, within a period of from 10 to 12 hours, often less, chlamydospores germinate and form a 3- or 4-cell promycelium on which sporidia arise from each cell (Fig. 1, B). As it appeared possible that the abnormality may have resulted from unfavorable environmental conditions, chlamydospores of cross 22A3 × 122B3

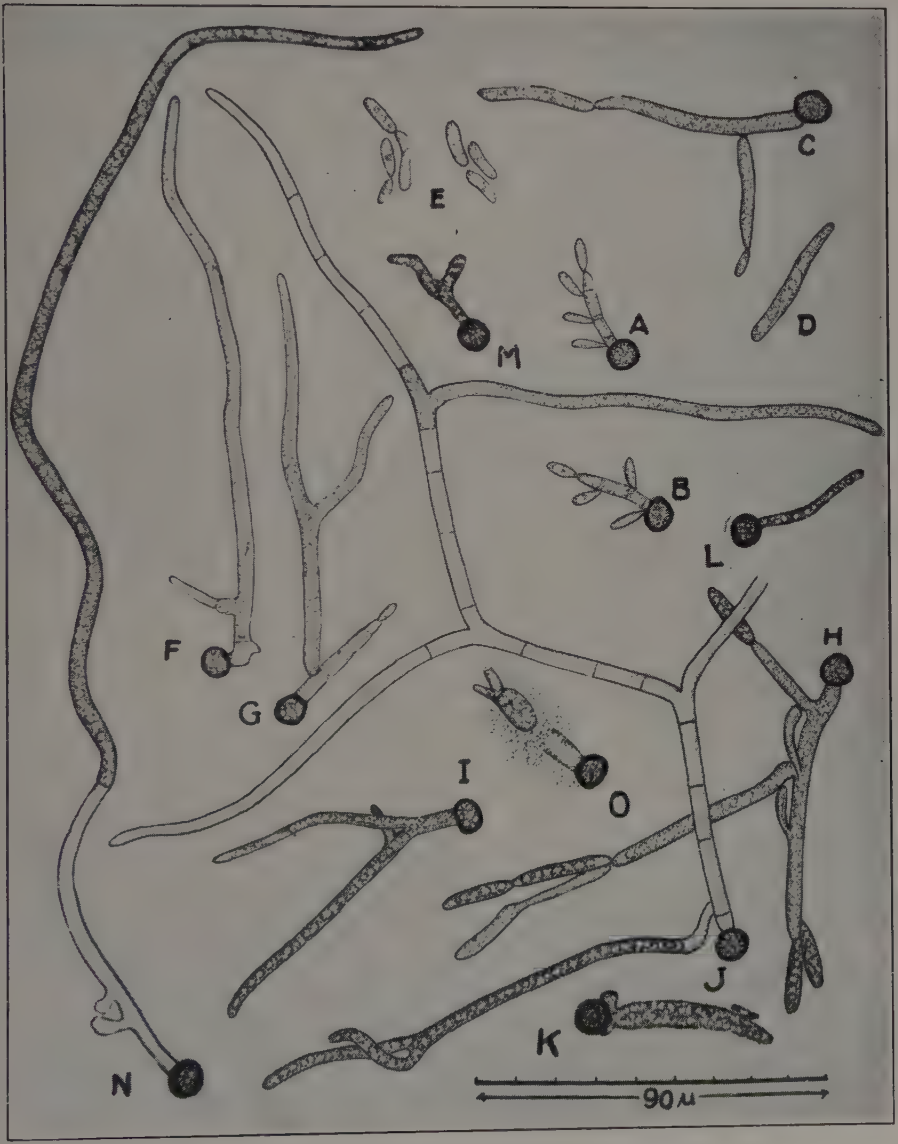

FIG. 2. Camera-lucida drawings of germinating chlamydospores of *Sphacelotheca sorghi*. A, B. Normally germinating chlamydospores from a non-lethal cross. C and F through O. Abnormally germinating chlamydospores from lethal cross $22A_3 \times 122B_3$. D and E. Variation in size of sporidia produced by chlamydospores from the same lethal cross.

were again germinated on carefully prepared potato-dextrose agar under the most favorable conditions of temperature, light, and moisture. Again, the germination was distinctly abnormal; it also was evident that the abnormality was being expressed in various ways. Chlamydospores from other monosporidial crosses germinated at the same time and under identical environmental conditions showed no evidence of abnormality.

Fully 90 per cent of the promycelia that develop from germinating chlamydospores of cross 22A3 × 122B3 disintegrate without forming viable sporidia. Most of the promycelia grow very slowly but may become very long, up to 200 μ, before they disintegrate (Fig. 2, N). Frequently, no septa or sporidia are formed, although abortive sporidia are not uncommon (Fig. 2, F, K, O). These may be short and globose, mycelioid, or they may be merely small papillae on the side of the promycelium; and all attempts to detach them by means of a micromanipulator were unsuccessful. Promycelia occasionally divided into 2 or more parts, forming a coenocytic, Y-shape structure, which in a few cases resembled the apical growth typical of germinating chlamydospores of the genus *Urocystis* (Fig. 2, I). Sometimes, a promycelium formed considerable atypical mycelium before disintegrating, as is illustrated in figure 2, J.

A small percentage of the chlamydospores from cross 22A3 × 122B3 germinated and produced nearly normal promycelia that formed viable sporidia and did not disintegrate (Fig. 1, C). In this group it was not uncommon to find promycelia with numerous cells, often 5, 6, or more. In a few cases some promycelial cells produced normal sporidia, while other cells in the same promycelium produced successive sporidia that disintegrated soon after they were fully formed. Occasionally, certain sporidia, after having been isolated from others, were observed to bud, form a colony of 20 to 30 individuals, then stop growing, and disintegrate.

Perhaps the most striking difference between lethal cross 22A3 × 122B3 and the normal crosses was the wide disparity in size between the atypical promycelia and sporidia of chlamydospores from the former and the normal ones from the latter. This is discussed more fully in a later paragraph.

The first evidence of lysis was the abnormally dull, granular appearance of the promycelium, apparently the result of the lysis or dissolution of the promycelial membrane at many points. Loss of turgidity usually followed, causing the promycelium to appear shrunken. As the lysis progressed, the granular appearance became more and more pronounced until the outline of the promycelium could be seen only with difficulty in the final stage. Another less frequent type of disintegration resulted from the rupture of the promycelial membrane due, apparently, to pressure developed from within. In such cases there appeared to be a localization of cell contents at the apices of promycelia and hyphae, which became swollen, finally rupturing and extruding granular bits of protoplasm into the surrounding area (Fig. 2, O). At no time did the chlamydospore wall disintegrate or appear in any way abnormal.

Other Lethal Crosses[4]

Germination tests were made with chlamydospores of several other crosses to find out whether they germinated normally. Lysis occurred in crosses 22A4 × 122B2, 122F2, and 22A1 × 22A4, although the percentage varied. Thus, the percentage of lysis in cross 22A4 × 122B2 averaged 50 to 60 per cent, 20 per cent in 122F2 and 22A1 × 22A4, and 90 per cent in 22A3 × 122B3.

Proof that Tendency Toward Lysis is Heritable

Several crosses were made by pairing monosporidial lines isolated from cross 22A3 × 122B3 with monosporidial lines from normal crosses and then inoculating into young sorghum seedlings in Petri dishes, after the method of Tyler (10). Of these crosses, only 3 caused infection and produced chlamydospores on the mature host. Crosses 150, 152, and 154, which produced chlamydospores, were examined for evidence of abnormal germination and each was found to average 20 per cent lysis. Inoculations were made also with the individual monosporidial lines that made up each of these crosses, but no chlamydospores resulted. Crosses between the normal monosporidial lines that were paired with the lines from cross 22A3 × 122B3 and other normal monosporidial lines produced only normally germinating chlamydospores. This evidence indicates that the lysis was attributable to a genetic factor or factors present in the haploid lines isolated from the lethal cross 22A3 × 122B3.

Size of Chlamydospores from Lethal and Normal Crosses

Chlamydospores were measured to check the observation that spores, promycelia, and sporidia of some of the crosses containing the lethal factor or factors were considerably larger than those of the normal crosses. The data obtained are given in table 1 and are based on measurements of 100 individuals from each cross listed. The chlamydospores were placed on thin potato-dextrose-agar drops on van Tieghem cells in Petri dishes when measured for any one character. The mean lengths and widths of promycelia produced by the lethal crosses were 71.4–20.6 μ × 4.4–2.9 μ, while those from the normal crosses were 24.4–18.2 μ × 2.9–2.8 μ. The mean lengths of primary sporidia from the lethal crosses were 18.9–9.6 μ, while the mean lengths of primary sporidia from normal crosses ranged from 10.8 to 9.7 μ. The mean diameters of chlamydospores from normal crosses ranged from 6.9 to 6.1 μ.

It is clear from table 1 that all of the characters measured were significantly larger in certain of the lethal crosses than in the normal crosses. In fact, the deviation in crosses 22A3 × 122B3 and 22A4 × 122B2 is so great as easily to exceed the limits of the species. In other crosses, however, there were no significant differences between lethal and normal crosses. This

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[4] For convenience, crosses in which there was considerable lysis are designated "lethal crosses."

TABLE 1.—*Comparative measurements on chlamydospores, promycelia, and primary sporidia of lytic and nonlytic crosses and field collections of Sphacelotheca sorghi*

Cross or collection	Chlamydospores (Diameter in microns)		Promycelia (Length in microns)		Promycelia (Width in microns)		Primary sporidia (Length in microns)	
	Range	Mean[a]	Range	Mean[b]	Range	Mean[c]	Range	Mean[c]
Lytic material								
22A3 × 122B3	8.1–6.3	7.1	207.2–26.6	71.4	6.0–2.8	4.4	58.9–9.8	18.9
22A4 × 122B2	....	......	77.0–23.8	40.6	....	......	30.8–8.4	17.1
22A1 × 22A4	....	......	....	......	....	......	22.6–8.4	12.6
122F$_2$	....	......	....	......	....	......	22.4–9.8	12.8
152	7.3–5.7	6.7	28.8–19.2	23.9	4.2–2.5	3.1	....	......
154	7.3–5.6	6.5	28.8–17.7	22.8	4.2–2.8	3.0	12.6–6.3	9.6
150	7.0–4.9	6.1	25.0–15.4	20.6	4.1–2.4	2.9	14.0–7.0	9.6
Nonlytic material								
201 Brown	7.3–5.3	6.7	30.4–19.8	24.4	....	......	....	......
Gray Brown A$_2$ × A$_3$	8.0–5.6	6.9	28.8–19.2	23.9	....	......	....	......
201 Gray	7.3–5.6	6.7	28.8–19.2	22.7	3.8–2.5	2.9	....	......
22A$_2$ × 22A$_3$	....	......	....	......	....	......	15.4–7.3	10.8
124 Gray B$_3$ × Brown A$_2$	7.3–4.9	6.5	....	......	....	......	....	......
124 Gray B$_2$ × Brown A$_4$	7.6–4.5	6.1	25.6–17.0	21.1	....	......	12.6–7.0	9.4
149 Gray	7.4–5.6	6.5	23.0–13.8	18.2	4.2–2.4	2.8	....	......
Field collection I	7.3–5.3	6.4	24.3–16.0	19.4	3.1–2.5	2.8	13.3–6.3	9.7
Field collection II	7.0–5.6	6.5	....	......	....	......	....	......

[a] Difference of means required for significance = 0.12.
[b] Difference of means required for significance = 3.42.
[c] Difference of means required for significance = 0.15.

may be explained by the fact that abnormal size appears to be correlated with the degree of lysis evident in the cross. Thus, cross 22A3 × 122B3 with an average of 90 per cent lysis showed the greatest plus deviation for all of the characters measured and cross 22A4 × 122B2, with 50 to 60 per cent lysis, ranked second. Crosses 150, 152, and 154, the result of pairing monosporidial lines from cross 22A3 × 122B3 with monosporidial lines from normal crosses, each averaged only 20 per cent lysis and were entirely normal in size of chlamydospores, promycelia, and sporidia. There are significant differences also between various normal collections, as would be expected in a species comprising many biotypes.

Solopathogenicity and Lethal Crosses

Solopathogenic lines, as defined by Christensen (2), are monosporidial lines capable of causing infection and forming chlamydospores when inoculated into the host alone. Solopathogenic lines have been reported in *Ustilago zeae* by Christensen (2), Eddins (3), Stakman *et al.* (9) and by Chilton[5] (1). Vaheeduddin[6] stated that 3 monosporidial lines of *Sphacelotheca sorghi* appeared to be solopathogenic, but he did not conclude definitely whether they were or were not. Chilton[5] concluded that solopathogenicity in *U. zeae* was associated with tendency to lysis. A study was made to determine if this was true also in *Sphacelotheca sorghi.*

In 1938, 76 monosporidial lines were isolated from the lethal cross 22A3 × 122B3 grown in potato dextrose broth and inoculated hypodermically into seedlings of Minnesota Amber Sorghum, 3 to 5 weeks old, growing in soil. In a preliminary experiment 27 of the lines were thus tested in the greenhouse, and later all were tested similarly in the field. All 76 lines were tested further by the method used by Tyler (10). In this method sorghum seeds were allowed to germinate on sterile moistened filter paper in Petri dishes. When the seedlings were 24 to 36 hours old they were inoculated by adding to each dish a sporidial suspension of the line to be tested. After remaining 36 hours longer in the dishes they were planted in the field. Only one monosporidial line, S18, ever caused infection and produced chlamydospores. This was in the first greenhouse experiment; but, in the several subsequent field tests, S18 failed to cause infection and the results therefore are inconclusive. It is possible that S18 lost its pathogenicity during the 6 or 7 months that intervened before it was tested a second time, as Christensen (2) found that some solopathogenic lines of *Ustilago zeae* lost this ability after a few months, although other lines remained solopathogenic after 4 years in culture.

The germination of the chlamydospores produced by line S18 was abnormal, but the percentage of lysis was comparatively low, being estimated at 10 to 15. Twenty-one monosporidial lines, isolated from chlamydospores produced by S18, were tested for solopathogenicity by hypodermically inoculating young sorghum seedlings in the field. All of these lines failed to produce chlamydospores.

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[5] See footnote 3.

[6] Vaheeduddin, S. Studies on the pathogenicity and genetics of some sorghum smuts. Unpublished Ph.D. thesis, University of Minnesota. 1936.

In the spring of 1939 an additional 200 monosporidial lines from cross 22A3 × 122B3 were tested in the field by inoculating them hypodermically into young sorghum seedlings of the very susceptible Minnesota Amber variety used in the previous tests. All of these failed to cause infection and produce chlamydospores.

Thus, of 301 monosporidial lines isolated from cross 22A3 × 122B3 and inoculated singly into susceptible sorghum seedlings in 1938 and 1939, S18 was the only one that produced chlamydospores; and 21 monosporidial lines isolated from these chlamydospores failed to cause infection. If a larger number of monosporidial lines from cross 22A3 × 122B3 had been tested, solopathogenicity might possibly have been found associated with lysis; but, on the basis of the evidence given above, such association appears not to be common in *Sphacelotheca sorghi.*

DISCUSSION

Abnormal germination in smut fungi may result from unfavorable environmental conditions (6). It also may be due to genetic factors in certain smut fungi[7] (1, 2, 5, 7, 8). Chlamydospores resulting from interspecific crosses sometimes germinate abnormally, as might be expected of wide crosses with sterility factors operating. It is clear, however, from the investigations of Chilton[7] and of the writer that lysis of promycelia regularly occurs in crosses between certain biotypes within *Ustilago zeae* and *Sphacelotheca sorghi.*

That the tendency toward lysis is due to genetic factors has been shown by making appropriate crosses. Chilton found that chlamydospores of these so-called lethal crosses tended to be larger than normal and that sporidia, if produced at all, tended to be diploid and solopathogenic. In his experiments, therefore, there appeared to be some association between abnormal size and behavior and abnormal diploidy.

In 8 lethal crosses within *Sphacelotheca sorghi* there also was a tendency toward the production of abnormally large chlamydospores, promycelia, and sporidia; and there seemed to be some association between the amount of deviation from the normal and the degree of lysis. No relation could be observed, however, between degree of lysis in crosses and tendency to produce diploid and solopathogenic sporidia. The writer tested more than 300 monosporidial lines from lethal crosses within *S. sorghi,* but in only one doubtful case was there evidence of solopathogenicity. Although still more tests would have been desirable, it seems clear that tendency toward lysis in *S. sorghi* is not necessarily associated with tendency toward diploidy where haploidy is the rule.

As concerns the importance of tendency toward lysis of promycelia of chlamydospores resulting from certain intraspecific crosses, it is evident that factors for lysis are likely to impose some limitations on the number of recombinations that may persist. In *Ustilago zeae* those combinations that are solopathogenic may be well able to persist because they are self-perpetuating. In *Sphacelotheca sorghi,* on the other hand, it seems likely that factors for lysis would operate more nearly as lethals, thus restricting the number of successful combinations within the species. More work should be done, however, before drawing final conclusions regarding the importance of lysis.

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[7] See footnote 3.

SUMMARY

A study was made of abnormal germination occurring in chlamydospores resulting from certain monosporidial crosses of *Sphacelotheca sorghi.*

The abnormality consisted in the disintegration or lysis of the promycelia prior to or after the formation of sporidia. Promycelia that produced sporidia and did not disintegrate were atypical in morphology and the degree of abnormality appeared to be correlated with the degree of lysis.

The tendency to lyse is governed by a genetic factor or factors, as it has persisted through 2 chlamydospore generations and is characteristic of only certain combinations of lines.

Chlamydospores, promycelia, and sporidia of certain lethal crosses were significantly larger than those of normal crosses; in some cases they easily exceeded the limits of the species.

Solopathogenicity is not necessarily associated with tendency toward lysis in *Sphacelotheca sorghi.* Of 322 monosporidial lines tested for solopathogenicity, only one produced chlamydospores, and then in only one test out of three.

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LITERATURE CITED

1. CHILTON, S. J. P. The occurrence of lysis in certain crosses of *Ustilago zeae.* (Abstract) Phytopath. **28**: 5. 1938.
2. CHRISTENSEN, J. J. Studies on genetics of *Ustilago zeae.* Phytopath. Zeitschr. **4**: 129–188. 1931.
3. EDDINS, A. H. Pathogenicity and cultural behavior of *Ustilago zeae* (Beckm.) Ung. from different localities. Phytopath. **19**: 885–916. 1929.
4. GOLDSCHMIDT, V. Vererbungversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violaceae* Pers.) Ein Beitrag zur Frage der parasitären Spezialisierung. Zeitschr. Bot. **21**: 1–90. 1928.
5. HOLTON, C. S. Hybridization and segregation in the oat smuts. Phytopath. **21**: 835–842. 1931.
6. KIENHOLZ, J. R., and F. D. HEALD. Cultures and strains of the stinking smut of wheat. Phytopath. **20**: 495–512. 1930.
7. LASKARIS, T. The occurrence of lysis in certain crosses of *Sphacelotheca sorghi.* (Abstract) Phytopath. **29**: 16. 1939.
8. RODENHISER, H. A. Heterothallism and hybridization in *Sphacelotheca sorghi* and *S. cruenta.* Jour. Agr. Res. [U. S.] **45**: 257–296. 1932.
9. STAKMAN, E. C., L. J. TYLER, G. E. HAFSTAD, and E. G. SHARVELLE. Experiments in physiologic specialization and nature of variation in *Ustilago zeae.* (Abstract) Phytopath. **25**: 34. 1935.
10. TYLER, L. J. Variation in *Sphacelotheca sorghi* (Link) Clinton. Minn. Agr. Exp. Sta. Tech. Bull. 133. 1938.

THERMAL DEATH POINT OF FUNGI IN RELATION TO GROWING CONDITIONS

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(Accepted for publication September 10, 1940)

It is expected that the thermal death point of fungi, like many other characters, is subject to the influence of growing conditions through changes in the physiological state of the organism. Such indication is given by divergent reports in literature regarding the thermal death point of the same species of fungus. However, no information is thus far available on experimental determinations of the nature and the extent of variation. In this paper, evidences are presented to show the relationship in conidia of *Colletotrichum* spp. between resistance to the lethal action of high temperature and environmental conditions under which conidia are produced.

MATERIAL AND METHODS

Monosporous strains of 3 species of *Colletotrichum*, namely, *C. nicotianae* Averna-Sacca strain 2, *C. gossypii* South. strain 10, and *C. glycines* Hori strain 10b were studied, because of their readiness to germinate and produce abundant conidia on artificial media. They were grown at 28° C. on 2 per cent potato-dextrose agar with pH at 6.0 throughout the experiments. The reaction of medium was adjusted by adding HCl and NaOH and was determined colorimetrically. Spores from young and vigorously growing cultures were suspended in beef-peptone broth, which was then diluted to uniform concentration for each trial. Thermal death point is expressed here as the temperature at which no spores germinate after the suspension has been exposed for a 10-minute period. For determination of thermal death point, ordinary methods for bacteriological study were followed. Spore suspension of the fungus to be tested was drawn up into sterilized capillary tubes several centimeters in length. Then the ends were sealed, and the tubes placed in a constant-temperature water bath at the desired temperature, with a deviation of not more than 0.2° C. in all cases. After a 10-minute exposure, the tubes were shifted immediately into cold water. Germination tests were made in van Tieghem cells, records being taken after 12 hours and again after 24 hours. Nonheated controls were maintained in all trials. All experiments were run in duplicate and most of them were repeated to corroborate the results.

EXPERIMENTAL RESULTS

Relation to Genetic Constitution

In the study of environmental effects in the biological field, the inherent condition of the organism naturally comes first into consideration. In order to determine whether variation exists between biotypes within a species, a number of monoconidial strains of *Colletotrichum* spp., which were considered genetically pure because of their single-nucleate origin, were compared under standardized conditions. From the results presented in table 1, it is obvious that the capability of withstanding high temperature characterizes each strain in a definite way, thus resembling many other physiological characters. In comparing the most thermally resistant strain with the most susceptible one in each species, a difference of 2 to 4° C. in thermal death point was found in two species, while *C. glycines* had a narrower range of variation.

The three species tested also show differences in resistance to the cell-destroying action of heat, *Colletotrichum nicotianae* being the least and *C. gossypii* the most resistant one. This was confirmed by experiments described later.

TABLE 1.—*Thermal death point of conidia of Colletotrichum spp. in relation to genetic constitution*

C. gossypii		*C. glycines*		*C. nicotianae*	
Strain	Thermal death point, in °C.	Strain	Thermal death point, in °C.	Strain	Thermal death point, in °C.
4	52	11a	51	1	50
2	51	9a	50	31b	50
1	50	12a	50	3	49
8	50	2a	49	2	46
10	49	10b	49		
5	48				

Relation to Incubation Temperature

After incubation for 15 days, sporulation occurred at temperatures ranging from 24 to 32° C. for *Colletotrichum glycines* and from 16 to 32° C. for the other two species. The thermal death points, as determined from those sporulated cultures, are recorded in table 2. Measurements of the diameter of colonies, taken 10 days afterward as a criterion of growth are also illustrated in graphic form in figure 1, A. In brief, the thermal tolerance of conidia increases with the rise of incubation temperature. Lower temperatures apparently render the conidia less tolerant. Although the

TABLE 2.—*Thermal death point of conidia of Colletotrichum spp. in relation to incubation temperature*

Incubation temperature, in °C.	Thermal death point, in °C.		
	C. gossypii strain 10	*C. glycines* strain 10b	*C. nicotianae* strain 2
16	48	—[a]	44
20	48	—[a]	44
24	48	48	45
28	49	49	46
32	49	49	46
36	—[a]	49	—[a]

[a] — indicates no sporulation.

variation thus induced is only 2° C. for *C. nicotianae* and 1° for the other two species, the persistence of such a variation in repeated experiments and its regular increase proportionally with incubation temperature indicate that the results obtained are significant.

Relation to Medium Reaction

The reaction of medium exerted a greater influence on the thermal resistance of *Colletotrichum* spp. than the incubation temperature (Table 3). Under the conditions of the experiments in which the pH of medium was modified, lethal temperatures for *C. gossypii* and *C. glycines* fall in the range from 49 to 51° C., and for *C. nicotianae,* from 45 to 48° C. It is possible that there is in existence more than one pH that provides suitable conditions to increase the thermal resistance of conidia. The actual location of such optimum pH will, however, vary with the organism dealt with. The measurements of 7 days growth are presented also in figure 1, B, for comparison.

TABLE 3.—*Thermal death point of conidia of Colletotrichum spp. in relation to medium reaction*

Initial pH of medium	Thermal death point, in °C.		
	C. gossypii strain 10	*C. glycines* strain 10b	*C. nicotianae* strain 2
3.6	49	49	47
4.2	49	51	—[a]
4.8	49	49	48
5.4	50	49	—[a]
6.0	49	49	45
6.6	49	49	—[a]
7.2	51	51	46
7.6	49	51	—[a]
8.4	49	49	47

[a] – indicates no test.

Relation to Nutrition

Besides the natural hosts, 5 different agar media, *i.e.,* 2 per cent potato-dextrose agar, Richard's medium, and Richard's with nitrogen, phosphorus, or potassium omitted, were selected for testing the thermal tolerance of conidia of *Colletotrichum* in relation to nutrition and substratum. The growth was equally good on potato-dextrose agar and complete Richard's medium. The medium from which nitrogen was omitted supported only very feeble growth, hence no sporulation resulted. On one or two of the other mentioned substrata, each of the 3 *Colletotrichum* species involved in this study also failed to produce spores. As shown in table 4, variation in thermal death point, as induced by nutritional difference, ranges from 46 to 50° C. for *C. nicotianae,* from 49 to 50° C. for *C. gossypii,* and from 50 to 51° C. for *C. glycines.* Except in *C. nicotianae,* where a radical lowering in thermal resistance is noticed in potato-dextrose agar in comparison with other media used, the nutritional conditions appeared to have little effect. In the other 2 species, the variation of only 1° C. seems of little significance.

TABLE 4.—*Thermal death point of Colletotrichum spp. in relation to nutrition*

Substratum	Initial pH of medium	Thermal death point, in °C.		
		C. gossypii strain 10	*C. glycines* strain 10b	*C. nicotianae* strain 2
Host	......	—[a]	49	—[a]
Potato-dextrose	6.6	50	50	46
Richard's	5.3	51	50	49
Richard's–K	5.4	51	—[a]	49
Richard's–P	6.2	50	—[a]	50

[a] – indicates no sporulation.

DISCUSSION

The thermal death point of fungi is generally studied for two main interests: As a reference to the practical application of heat in controlling disease caused, and as a physiological problem in the developmental history of the organism. It is, however, difficult to evaluate the data thus obtained before the physiological aspects of the so-called "thermal death point" itself are fully understood. Although the results as presented in this paper clearly show that the thermal death point is not a static phenomenon, being influenced considerably by both external and internal conditions, the question may arise as to its constancy, even under uniform environment. In the different experiments mentioned above, all but one of the factors are kept relatively uniform. Therefore, corresponding figures can be taken out one by one from the above tables when all the controlled factors are comparable. These figures thus taken out are compiled in table 5 for comparison. Irrespective of differences of one degree, which probably are induced by slight variations in pH and also the composition of different batches of potato-dextrose agar, the similarity of figures determined at different times for the particular strain in each species indicates that the thermal death point is a relatively constant character.

TABLE 5.—*Thermal death point of conidia of Colletotrichum spp. determined at different times for material grown on the same medium and at the same incubation temperatures*

Source of figures	pH of medium	Thermal death point, in °C.		
		C. gossypii strain 10	*C. glycines* strain 10b	*C. nicotianae* strain 2
Table 1	6.0	49	49	46
Table 2	6.0	49	49	46
Table 3	6.0	49	49	45
Table 4	6.6	50	50	46

Nevertheless, even under more or less constant experimental conditions, the resistance of conidia of *Colletotrichum* to high temperature may be considerably altered by environmental factors. In *C. nicotianae* the thermal death point may cover a wide range, from 44 to 50° C. In nature, the interaction of climatic and numerous other factors is expected to be more complicated and variable. Consequently, the environments under which the fungi grow and fructify may exert even greater influence than the present results indicated on their ability to withstand severe weather, and hence on the chances of their perpetuation. This aspect may also become a limiting factor in the distribution of physiologic races of fungi. For instance, in cereal rusts the possibility of oversummering or overwintering is important in certain localities in considering epidemics. In other words, the probability for a certain race to establish itself in northern latitude may be determined by its ability to survive cold winter, and in southern latitude

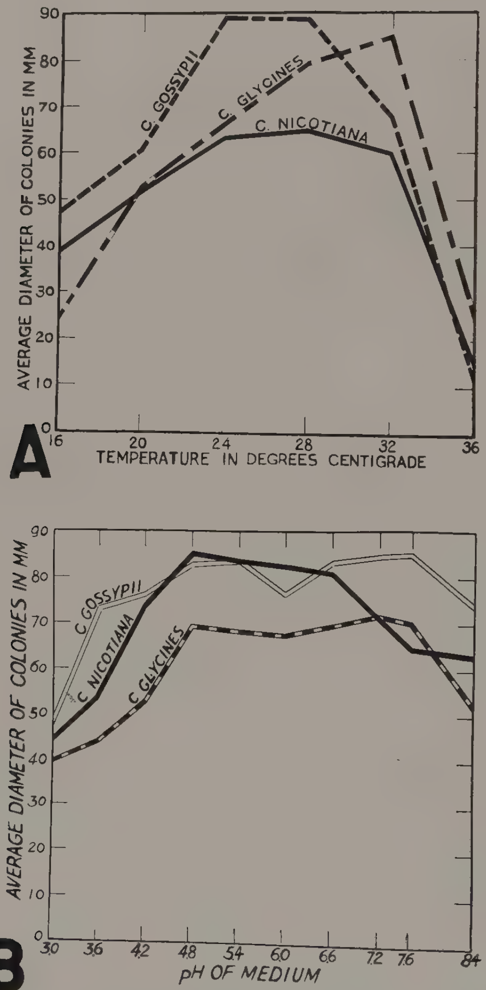

FIG. 1. A. Effect of temperature on the growth of *Colletotrichum* spp. B. Effect of pH of medium on the growth of *Colletotrichum* spp.

by its ability to survive hot summer. The problem of oversummering or overwintering is, however, not merely related to the temperature in summer or in winter; but equal, if not more, weight must be considered as to the environmental conditions of the previous growing period.

From the practical point of view, the fact that genetic variations for thermal death point exist between species, and even between strains within a species, is important. If the thermal death point is variable, a fixed schedule recommended for general use in eliminating a certain fungus, borne inside the seed or other plant parts, as in the case of cotton anthracnose, may not always give satisfactory results, as would be expected. Failure after applying such a schedule might be due to the occurrence of a particular strain of the pathogen highly tolerant to the destructiveness of the thermal effect.

Concerning the relation of incubation temperature and medium reaction to thermal death point, it seems justifiable to make a generalized statement that the conidia produced under the environments most suitable for growth stand the best chance for resisting high temperature. Being vigorously growing and healthy, the organism is expected to be more tolerant to disturbance by the accelerating action of heat. This is especially true in the cases conditioned by incubation temperature. In comparing figure 1, A, and table 2, the incubation temperatures at which conidia with the highest thermal resistance are produced are in all cases located at and near the optimum temperatures for growth. Of course, the alternative explanation may be that heat resistance is simply a matter of acquired adaptation, as the organism has grown under higher temperatures long enough to be able to withstand them better. A similar relationship, however, is also evident between thermal death point of conidia and reaction of medium. As shown in figure 1, B, the graphs representing the pH relation of growth in *Collectotrichum glycines* and *C. gossypii* are both of bimodal type. There exist also two modes where the thermal resistance of conidia is increased. Taking *C. glycines* for illustration, the two modes in the growth curve, one at pH 4.8 and the other at pH 7.2, are in close accordance with the regions showing highest thermal resistance, which are 4.2, 7.2, and 7.6 in pH scale (see Table 3). For *C. gossypii,* the corresponding figures are 5.4 and 7.6 in the former condition and 5.4 and 7.2 in the latter. The occurrence of slight disagreement probably is due to the fact that the intervals in pH used are too wide to show the real optima. In the case of *C. nicotianae,* a somewhat normal curve correlates the medium acidity and growth rate, with mode at pH 4.8. It is also at the same pH that the death temperature is highest.

Regarding the alteration of the thermal death point of *Colletotrichum* by modifying nutritional conditions during the previous growing period, the influence is apparently not so profound. The results so far obtained are only enough to indicate the possible occurrence of such an alteration. But it is far from adequate to answer whether the thermal resistance is correlated with vigor of growth, or with any particular chemical elements in the substratum. Further studies are necessary to throw more light on this problem.

SUMMARY

Three species of *Colletotrichum* were selected in comparing their thermal death points under different growing conditions by exposing the conidial suspension at constant temperatures for a period of 10 minutes. General technic for determining the thermal death point of bacteria was followed.

Alteration in thermal resistance was found among monoconidial strains within a species, and under the influence of incubation temperature, pH of medium, and nutritional conditions. Among the three species tested, *C. nicotianae* was most affected by the environmental factors, its thermal death point varying from 44 to 50° C. For *C. gossypii* and *C. glycines,* the ranges of variation were 48 to 52 and 48 to 51 degrees C., respectively.

Regarding the effects of incubation temperature and reaction of medium, a general relation was noticed that the conditions most suitable for fungus growth usually render the conidia produced most resistant to the destructive thermal effect. The variation induced by nutritional differences is less profound, and its cause is hardly explained on the basis of the results secured thus far.

As the environmental conditions in nature are expected to be much more variable, the variability of thermal resistance of fungi is considered to be important in the practical application of heat in controlling plant diseases caused by them, in their survival and perpetuation in certain localities, and in the determination of the distribution of physiologic races.

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FUNGI ASSOCIATED WITH STAIN IN CHEMICALLY TREATED GREEN LUMBER

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(Accepted for publication August 2, 1940)

INTRODUCTION

During the past 10 years the use of effective chemical treatments to protect lumber from fungus stains during the air seasoning period has become general, particularly in the Southern States.[1] The general effectiveness of the treatments in commercial use is even more than was anticipated during their development. Occasionally, however, mills have reported severe staining of chemically treated lumber. Some of these failures were obviously due to poor handling practices but the possibility existed that certain staining fungi, possibly of little importance on untreated wood, were more tolerant to the toxics used than were fungi known to cause important staining of untreated wood.[2] The present study was made to determine what fungi were associated with occasional stain developing in lumber treated with the common commercial chemicals used to prevent staining.

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[1] Scheffer, T. C., and R. M. Lindgren. Stains of sapwood and sapwood products and their control. U. S. Dept. Agr. Tech. Bull. 714. 1940.

METHODS

The general methods used were the same as those previously reported[2] in determining the fungi associated with stain in untreated lumber, the only difference being that part of the test lumber (mostly 1 × 4 × 48 inches) was dipped in a chemical solution for approximately 5 seconds before seasoning. The chemicals used were Lignasan (6.25 per cent ethyl mercuric chloride) 0.31 per cent and Dowicide P (a 1–1 mixture of sodium tetrachlorophenolate and sodium 2-chloro-orthophenylphenolate), 1.03 per cent, representing the two groups, organic mercuries and chlorinated phenols, in general commercial use. The concentrations used were approximately those used in commercial mill operations.

The treated and the untreated boards were piled randomly on dry cross sticks in the same pile. Pine (shortleaf, loblolly, and longleaf) and hardwoods (mostly red gum) usually were piled separately.

Tests were made chiefly at one mill in northern Louisiana during most months in 1938 and March, August, and November, 1939. A few tests were made in southeastern Louisiana and northern Mississippi. The preponderance of tests at one mill probably was no hindrance to good sampling because previous work[2] showed that there was little difference in staining floras in widely separated localities in the southern States.

RESULTS

The tests were complicated by two difficulties: that of securing any stain in carefully selected treated hardwoods and that of getting appreciable stain in mercury-treated pine without complicating molding. In very few tests was usable stained material secured in nontreated lumber and that treated with both the chemicals. Because of this the comparisons are all made between each treatment and the nontreated controls accompanying that treatment when usable stained material was secured in the treated wood.

In table 1 are shown the relative frequencies with which the various fungi were isolated.

Fungi Isolated from Pine. In both treated and nontreated material the stain was attributable mainly to *Ceratostomella pilifera* (Fr.) Winter, with *C. ips* Rumbold second in importance. *Diplodia natalensis* Evans and *D.* sp. are sometimes important;[2] but, since they were practically absent on pine in the present tests, their place in the staining of treated pine remains

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[2] Verrall, A. F. Relative importance and seasonal prevalence of wood-staining fungi in the Southern States. Phytopath. 29: 1031–1051. 1939.

TABLE 1.—*Frequency of isolation of different fungi from stained lumber treated with Lignasan or Dowicide and from untreated stained lumber*

Fungus and kind of lumber	Per cent of stained samples yielding fungi listed			
	Lignasan-treated	Non-treated	Dowicide-treated	Non-treated
From pine lumber:				
Ceratostomella pilifera	71	71	87	73
C. ips	11	13	13	14
Diplodia sp.	1	1	4	1
D. natalensis	0	1	0	0
Alternaria spp.	6	4	1	4
Cadophora spp.	4	0	5	0
Ceratostomella sp.	0	1	0	1
C. pluriannulata	0	1	0	1
Cladosporium spp.	3	0	0	0
Endoconidiophora moniliformis	0	0	1	0
Graphium rigidum	0	4	0	3
Helminthosporium geniculatum	3	2	1	2
Pullularia spp.	4	0	0	0
Dark, undetermined[a]	11	19	3	19
Nonstaining[b]	100	99	92	99
Number of samples	72[c]	90	135[d]	91
From hardwood lumber:				
Ceratostomella pluriannulata	37	71	23	77
Endoconidophora coerulescens	67	44	69	38
Graphium rigidum	27	48	8	35
Diplodia natalensis	0	19	0	21
Alternaria spp.	0	2	0	0
Cadophora spp.	0	11	0	27
Ceratostomella sp.	0	2	0	4
Endoconidiophora moniliformis	2	29	15	42
Dark, undetermined[a]	8	2	8	0
Nonstaining[b]	82	90	85	92
Number of samples	49[e]	63	13[f]	26

[a] Probably includes some of the other fungi listed, but, if so, they were obscured by other organisms.

[b] Includes bacteria and yeasts as well as higher fungi.

[c] Includes 7 tests at 3 mills.

[d] Includes 8 tests at 4 mills.

[e] Includes 7 tests at 1 mill.

[f] Includes 3 tests at 1 mill.

undetermined. Of the stain that occurred despite Lignasan treatment, the proportion chargeable to the various fungi was nearly identical with that in the untreated. The Dowicide appeared somewhat less effective against *C. pilifera* and relatively more effective against the molds and dark, undetermined group. In view of the possible obscuring of *C. pilifera* by the dark, undetermined fungi and the molds, conclusion that there was more of it in the stained Dowicide-treated wood would be risky.

Fungi Isolated from Hardwoods. Both treatments reduced the proportion of the stain caused by *Ceratostomella pluriannulata* Hedgc., *Graphium rigidum* (Pers.) Sacc. and *Diplodia natalensis,* particularly the last, which was never isolated from treated material. The fungi that, because of infrequency of isolation or poor staining ability, are considered of minor importance on nontreated wood[3] are apparently of even less importance on treated wood. *Endoconidiophora coerulescens* Münch was the one important fungus that gave definite evidence of being less easily controlled by the treatments than the staining fungi in general, but it may also be noted that the dark, undetermined fungus group appeared less easily controlled than the corresponding group on pine. Both *G. rigidum* and *C. pluriannulata* were isolated in appreciably greater numbers from the Lignasan-treated wood than from the Dowicide-treated. *D. natalensis* was not isolated from treated wood, even in individual tests in which it was isolated from as high as 86 per cent of the nontreated samples.

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[3] See footnote 2.

Although but 2 chemicals were extensively tested, preliminary tests with ethyl mercuric phosphate, sodium tetrachlorophenolate, and sodium pentachlorophenolate indicate that there are no differences in staining floras occurring on lumber treated with these and the 2 chemicals extensively tested. From a small amount of borax-treated hardwood and soda-treated pine, *Diplodia natalensis* was isolated a few times, although it was never isolated when any other treatments were used.

DISCUSSION

From the isolation results here reported it seems that there is not a very specialized stain flora on pine lumber treated with the important commercial stain-control chemicals of the organic mercurial and chlorinated phenol types. Although there are some differences between the floras from treated and nontreated pine, these differences seem insufficient to explain the occasional cases in which extensive staining of treated lumber occurs. Also, there is little probability that tolerant forms of the important species staining pine are being built up to an appreciable extent with the general practice of chemical treatment. No indication has been observed that control has become more difficult in either pine or hardwoods. Observations indicate that the vast bulk of stain inoculum is produced on nontreated wood, and that when severe staining of treated wood occurs the cause usually can be traced to some poor handling practice and not to direct failure of the treatments.

Endoconidiophora coerulescens is probably the most important staining fungus on hardwoods, and chemical treatment seems less effective against this fungus than against the other staining fungi. However, with hardwood lumber cut from stain-free logs, chemical control is relatively easily attained. This is shown by general observations and by the fact that in the present tests over two-thirds of the treated hardwood lumber remained stain-free under severe seasoning conditions; and, where stain occurred, it usually was limited to small areas on the treated boards but entirely covered the nontreated. In contrast to this, some stain was easily secured on most pieces of treated pine under the severe conditions of these tests. Because of the relative ease of stain control with hardwoods and the observation that most stain in treated hardwoods results from infections in the logs prior to treatment, it seems that possible resistant stains would be built up slowly.

Too long storage of logs, allowing infection of pine logs particularly with fungi carried by *Ips* beetles, and infection of hardwood logs at the ends and with fungi carried by ambrosia beetles and too long an interval between the milling operation and the chemical treatment appear to be practices responsible for many cases of treatment failure in the South. Delayed treatment is becoming particularly important with the increased use of portable saw mills whose outputs are frequently trucked to a concentration yard for treatment and seasoning. Oftentimes the interval between milling and dipping at the concentration yard is sufficient to allow the staining fungi to penetrate deeper into the wood than does the subsequent chemical treatment. The effect of the above and other handling practices on stain development has been discussed recently by Scheffer and Lindgren.[4]

Protracted periods of weather unfavorable for drying may cause general failure of chemical treatments but this cause appears to be a less frequent one than the poor handling practices.

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COMPARATIVE STUDIES OF SUGAR-BEET AND POTATO ISOLATES OF RHIZOCTONIA SOLANI[1]

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(Accepted for publication Sept. 10, 1940)

INTRODUCTION

Previous investigations[2] have demonstrated that isolates of *Rhizoctonia solani* from stem-lesions of older plants and sclerotia on tubers of potato (*Solanum tuberosum*) are nonpathogenic to sugar-beet roots, whereas isolates from sugar-beet (*Beta vulgaris*) and many isolates from stolon lesions of potato are pathogenic.

The present study was made to ascertain whether or not differences existed among the 4 types of isolates with respect to morphology, physiology, and pathogenicity.

EXPERIMENTAL RESULTS

Morphology

To determine whether the hyphal diameter of some potato isolates differed from that of a group of sugar-beet isolates, 10 isolates from each group were studied. Of the potato isolates, 4 were obtained from stem lesions of older plants and 6 from sclerotia on tubers.

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[4] See footnote 1.

[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1783 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] LeClerg, E. L. Pathogenicity studies with isolates of *Rhizoctonia solani* from potato and sugar beet. Phytopath. 31: 49–61. 1941.

The isolates were grown on potato-dextrose agar in Petri dishes for 5 days at room temperature. The hyphae were then mounted in a 10 per cent aqueous solution of glycerine on glass slides. By means of a projection apparatus the images were thrown on a white background and measured. The diameters of 200 young hyphae of each isolate were determined, measurements being made to the nearest tenth of a millimeter with a vernier-caliper. Unconscious selection of hyphae was avoided by measuring all hyphae encountered in passing from one end of the mount to the other.

Table 1 gives the mean diameters of hyphae of each of the 20 isolates.

TABLE 1.—*Summary of differences in mean hyphal diameters of 10 potato and 10 sugar-beet isolates of Rhizoctonia solani*

Potato isolates[a]			Sugar-beet isolates[a]		
Number	Place of isolation	Diameter	Number	Place of isolation	Diameter
	Stem-lesion isolates	*Microns*			*Microns*
P–100	Baarn, Holland	9.5	SB–42	Michigan	7.8
P–116	Baton Rouge, La.	10.4	SB–39	do	8.0
P–90	Charlottetown, Canada	11.1	SB–45	California	8.2
P–20	Dilworth, Minn.	11.4	SB–49	Michigan	8.2
			SB–18	Mankato, Minn.	8.7
	Sclerotial isolates		SB–28	do	8.8
P–85	Quebec, Canada	9.2	SB–13	Chaska, Minn.	9.0
P–36	Amherst, Mass.	10.0	SB–33	Oslo, Minn.	9.0
P–49	Alliance, Nebr.	10.3	SB–43	Ohio	9.0
P–105	Chevaliszewo, Poland	10.7	SB–50	E. Grand Forks, Minn.	10.7
P–38	Dove Creek, Colo.	10.8			
P–27	Laramie, Wyoming	11.1			

[a] Difference required for significance = 0.2 micron.

The stem lesion and sclerotial isolates from potatoes, as groups, do not differ materially in hyphal diameters. All but one of the sugar-beet isolates are definitely smaller than any of the potato isolates.

Physiology

Comparative experiments were made to study the radial growth of 20 isolates from each of the 4 groups on different artificial media and to determine the optimum temperature for radial growth.

Effect of Medium on Radial Growth. The radial growth, as measured by diameter of colony, was studied on 2 different media. The averages given for potato-dextrose agar are determined from duplicate Petri dishes in each of 2 experiments; only 1 test, consisting of triplicated Petri dishes, was made on the high-nitrogen agar.[3] The data were analyzed by the analysis of variance.[4]

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[3] The high-nitrogen agar was prepared according to the formula given by Floyd A. Heck in "A Study of the Nature of the nitrogenous compounds in fungous tissue and their decomposition in the soil," as published in Soil Sci. 27: 1–48. 1929.

[4] Fisher, R. A. Statistical methods for research workers. Oliver and Boyd, Edinburgh and London. Fourth Edition, 1932.

From the data in table 2 it appears that the radial growth of the 4 groups of isolates is significantly different. On both media, the sugar-beet group of isolates grew fastest, followed in order by the stolon-lesion, stem-lesion, and sclerotial groups from potatoes.

TABLE 2.—*Radial growth of sugar-beet isolates and of potato stolon-lesion, stem-lesion, and sclerotial isolates of Rhizoctonia solani, as groups, on potato-dextrose and high-nitrogen media*

Group of isolates	Number of isolates tested	Radial growth (mm.) on Potato-dextrose	High nitrogen	Average
Sugar-beet	20	40.6	35.0	37.8
Potato				
Stolon-lesion	20	38.6	28.8	33.7
Stem-lesion	20	32.1	17.4	24.7
Sclerotial	20	24.2	13.5	18.8
Required difference	.....	1.3	0.8	....

Effect of Temperature on Radial Growth. The influence of temperature on radial growth of 20 isolates from each of the 4 groups was studied at 20, 25, and 30 degrees C. The data presented are averages of duplicate cultures of each isolate at each temperature.

From the data in table 3 it appears that 30° C. was the optimum temperature for all sugar-beet isolates except one (SB–33). This isolate grew slightly faster (1.5 mm.) at 25° C. than it did at 30° C. It may be that with further repetition this difference might be changed in favor of the higher temperature.

TABLE 3.—*Optimum temperature of sugar-beet isolates and of potato, stolon-lesion, stem-lesion, and sclerotial isolates of Rhizoctonia solani as groups, as indicated by the number of isolates with maximum radial growth at the indicated temperatures*

Group of isolates	Number of isolates tested	Number of isolates with optimum temperature at 20° C.	25° C.	30° C.
Sugar-beet	20	0	1	19
Potato				
Stolon-lesion	20	0	17	3
Stem-lesion	20	1	19	0
Sclerotial	20	0[a]	20	0

[a] One isolate grew equally well at 20° and 25° C.

Of the 20 stolon-lesion isolates tested, all except 3 had an optimum temperature at 25° C. The potato-stolon-lesion isolates P–151, P–162, and P–174 had an optimum at 30° C. It is known[5] that P–162 causes dry-rot canker of sugar beets, and the dry-rot-canker isolates that have been tested have all had an optimum at 30° C. Preliminary inoculations indicate that isolates P–151 and P–174 probably are dry-rot-canker isolates, also, since they produced spotting of older sugar-beet roots typical of the early development of dry-rot canker.

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[5] LeClerg, E. L. Studies on dry-rot canker of sugar beets. Phytopath. 29: 793–800. 1939.

Nineteen of the 20 stem-lesion isolates had optima at 25° C. and one at 20°. Of the sclerotial group, all except one grew best at 25° C. This one (P–30) grew equally well at 20° and 25° C.

Thus it appears that the stem-lesion and sclerotial isolates from potatoes all had an optimum at 25° C. or below, whereas 30° C. was optimum for 19 of the 20 sugar-beet isolates. Likewise, the potato stolon-lesion isolates, which do not cause dry-rot canker of sugar beets, have an optimum temperature for growth on artificial media at 25° C.

Pathogenicity

Damping-off experiments were made to determine the pathogenicity of 20 isolates of each of the 4 groups, using sugar beets, beans, peas, and cabbage as hosts. Since all of the 80 isolates were tested on each host at different dates, no comparison can be made of the virulence of the groups with regard to the different hosts.

Inoculum was produced on a substratum consisting of sterilized oats and wheat grains, mixed with steamed soil, and allowed to stand 3 or 4 days before the seed was planted. The control pots were treated in the same manner, except only steamed grains were mixed with the soil. Each test consisted of 5 replications and the pots were arranged as a randomized block. The combined data for the potato isolates obtained, respectively, from stolon-lesions, stem lesions, and sclerotia were analyzed by the analysis of variance. In making this statistical reduction of the data for emergence, expressed as percentages for each host, the data for sugar-beet isolates were not included with the other 3 groups, since they comprised so many zero percentages. It was obvious that the sugar-beet isolates as a group were more virulent than any of those from potato.

The relative virulence of the isolates, as indicated by emergence, on the

TABLE 4.—*Reaction of seedlings of sugar-beets, beans, peas, and cabbage to sugar-beet isolates and to potato, stolon-lesion, stem-lesion, and sclerotial isolates of Rhizoctonia solani, as groups, in experiments made with steamed soil in the greenhouse. Data analyzed according to source of isolates*

Groups of isolates	Number of isolates tested	Percentage emergence				Infection of beans as estimated from surviving plants[b]
		Sugar beets	Beans	Peas	Cabbage	
Sugar-beet	20	9	27	13	28	1.38
Potato						
Stolon-lesion	20	74	84	63	69	1.18
Stem-lesion ...	20	82	91	70	70	0.86
Sclerotial	20	82	92	80	72	0.74
Required difference[a] ...	......	3	3	5	5	....

[a] For potato groups.

[b] 0 = no infection; 2.0 = severe infection.

4 hosts is given in table 4. It is apparent that the isolates of the sugar-beet group were markedly more aggressive than those of any of the 3 potato groups of isolates on all of the 4 hosts. The isolates from stolon lesions, as a group, reduced emergence of sugar beets, beans, and peas significantly more than either the stem-lesion or sclerotial groups. No significant differences in such effects were found between the stem-lesion or sclerotial groups, except that the former was slightly more aggressive on peas. On cabbage the 3 potato groups were about equally virulent.

After stand counts were made of the beans, the surviving plants were pulled and classified as to degree of injury on the underground stem. From table 4, it appears that the sugar-beet and potato stolon-lesion isolates, as groups, were more virulent than the stem-lesion or sclerotial groups of potato isolates.

SUMMARY

Comparative studies of morphology, physiology, and pathogenicity were made of isolates of *Rhizoctonia solani*, considered representative of the following groups: isolates from sugar beets; and potato isolates obtained from stem lesions of older plants, stolon lesions, and from sclerotia on tubers.

Four isolates from potato stem lesions and 6 from sclerotia did not differ significantly in hyphal diameters. Nine of the 10 sugar-beet isolates studied were significantly smaller than any of the potato isolates.

The sugar-beet isolates grew faster on potato-dextrose and high-nitrogen media than any of the 3 types of potato isolates. Of the potato isolates, those obtained from stolon lesions grew fastest, those from stem lesions next, and those from sclerotia slowest.

From a study of the optimum temperature for radial growth on potato-dextrose agar of 20 isolates from each of the 4 groups, it was found that the mature-plant and sclerotial isolates from potatoes all had an optimum at 25° C. or below, whereas 30° C. was optimum for 19 of the 20 sugar-beet isolates. Likewise, those potato-stolon isolates that do not cause dry-rot canker of sugar beets have an optimum of 25° C. The stolon-lesion isolates capable of causing dry-rot canker of sugar-beets grew best at 30° C.

Sugar-beet isolates, as a group, caused a higher percentage of damping-off of sugar beets, beans, peas, and cabbage than any of the 3 groups of potato isolates. Those obtained from potato-stolon lesions, as a group, reduced stands of sugar beets and peas more than potato stem-lesion or sclerotial isolates. The isolates from potato-stem lesions and from sclerotia, as groups, did not differ significantly in their ability to cause damping-off of the hosts tested, although the former group of isolates was more aggressive on peas. On cabbage, the 3 types of potato isolates did not differ significantly in reduction of emergence of cabbage.

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LONGEVITY OF SCLEROTIA OF THE STEM–ROT FUNGUS LEPTOSPHAERIA SALVINII[1]

E. C. Tullis and E. M. Cralley[2]

(Accepted for publication August 12, 1940)

In a preliminary report by the writers[3] on laboratory and field studies on the development and control of stem rot of rice caused by *Leptosphaeria salvinii* Catt. (*Sclerotium oryzae* Catt.), it was stated that rotation seemed impracticable as a control measure, since the sclerotia of the fungus apparently were long-lived in the soil. In view of the limited information available on this subject, experiments were started to obtain definite data on the longevity of the sclerotia.

In October, 1933, experiments were started at the Rice Branch Experiment Station, Stuttgart, Arkansas, to determine the longevity of the sclerotia under various field and laboratory conditions. Eight concrete forms, 3 ft. square and 2 ft. high, open at top and bottom, were placed in the soil to a depth of 1 ft., and the soil tamped around the inner and outer edges. Rice straw, heavily infested with sclerotia, was then buried at a depth of 4 to 6 in. in 7 of the 8 forms. In the 8th form the straw was put on top of the soil. Infested straw also was mixed with soil and sealed in $\frac{1}{2}$-gal. fruit jars, buried near the forms. The tops of 6 of the forms were covered with 200-mesh copper cloth on tight-fitting, wooden frames. This metal cloth prevented the soil inside the forms from being contaminated with sclerotia from external sources. Two of the forms were covered with solid tops, and composition roofing was used to make the tops water tight. Soil temperatures were recorded for 1938 and 1939 only. The average, mean, monthly soil temperatures in °F, were: for January, 43.1, February, 45.4, March, 52.2, April, 60.9, May, 69.9, June, 78.5, July, 82.2, August, 82.0, September, 71.9, October, 63.5, November, 48.6, and December, 43.1. The average yearly precipitation from 1933 to 1939 was about 50 in. Samples of soil were removed at yearly intervals from 1935 to 1939 and the sclerotia recovered were surface-sterilized in mercuric chloride (1–1000) for 1 min., washed in sterile water, and then plated on corn-meal agar to determine whether they were viable.

RESULTS

The average percentage of viable sclerotia recovered is shown in table 1 and represents viability tests on from 75 to 250 sclerotia recovered from each treatment.

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[1] Research Paper No. 687, Journal Series, University of Arkansas, Fayetteville, Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station. Cooperative investigations of the Division of Cereal Crops and Diseases and the Arkansas Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Assistant Plant Pathologist, Arkansas Agricultural Experiment Station.

[3] Tullis, E. C., and E. M. Cralley. Laboratory and field studies on the development and control of stem rot of rice. Ark. Agr. Exp. Stat. Bull. 295. 1933.

TABLE 1.—*Percentage of viable sclerotia recovered from soil artificially infested with sclerotia of the stem-rot fungus Leptosphaeria salvinii in 1933*

Sclerotia in infected straw		Viable sclerotia in				
Lot No.	Treatment	1935	1936	1937	1938	1939
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	Buried in soil, forms covered with copper cloth	32.0	1.3	0.0	5.5	0.0[a]
2	do	29.0	4.6	7.5	2.3	4.7
3	do	35.0	10.4	0.5	2.4	5.9
4	do	12.0	0.0	0.0	0.0[a]	0.0[a]
5	do	24.0	1.3	2.5	5.5	8.0
6	On surface of soil, forms covered with copper cloth	5.0	4.6	22.0	1.6	0.0
7	Buried in soil, forms covered with roofing	8.0	1.2	0.0	3.8	0.0
8	do	20.0	5.3	1.5	0.7	3.6
9	Sealed in 2-quart fruit jars and buried in soil	25.0	0.0[b]	0.0[b]	0.0[b]	0.0[b]

[a] Copper cloth broke, so no further samples were taken.
[b] Strong odor of acetic acid in jars.

From 5 to 35 per cent of the sclerotia plated were viable at the end of 2 years, and, in 4 of the forms, a small number of viable sclerotia were recovered at the end of the 6th year. In lots 1 and 7 viable sclerotia were recovered in 1938 but not in 1937. No doubt viable sclerotia would have been found in 1937 if a larger number of sclerotia had been tested.

The question as to whether the viable sclerotia recovered in 1939 had been buried in the soil since 1933 cannot be definitely answered, because the formation of sclerotia on organic matter in soil in Petri-dish cultures has been observed, and this process possibly may take place also in the soil under field conditions. In common field practice, rice stubble stands in the fields following the harvest. It may be 2 years before it rots, and the stubble crowns embedded in the soil may persist for even longer periods. Therefore, the fungus possibly may form new sclerotia in parts of the plants below the soil surface for a period of 2 or more years. If, therefore, a large amount of straw bearing sclerotia, or other organic matter, is turned under, it is obvious that the fungus may persist in the soil for much longer periods than has been commonly believed possible. Additional information on the longevity of sclerotia in stubble and in soil under different environmental conditions, is presented in table 2.

In these tests the sclerotia from stubble in the field also remained viable for 2 years, and under less variable conditions in the laboratory for 29 but not for 39 months. Viable sclerotia were recovered from soil uncultivated for 3 years. The results shown in table 2 are in general agreement with those of other investigators. Park and Bertus[4] showed that the sclerotia

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[4] Park, M., and L. S. Bertus. Sclerotial diseases of rice in Ceylon. II. Sclerotium oryzae. Ann. Roy. Bot. Gard. Peradeniya **6**: 355–356. [The Ceylon Jour. Sci., Sect. A.] 1932.

TABLE 2.—*Studies on the longevity of sclerotia of Letosphaeria salvinii under different environmental conditions*

Treatment	Sclerotia	
	Tested	Viable
	Number	*Per cent*
Stubble in field 12 months	75	25.0
Stubble in field 25 months	100	3.0
Stubble in laboratory 15 months	50	44.0
Stubble in laboratory 29 months	50	4.0
Stubble in laboratory 39 months	50	0.0
Stubble in field 8 months and in laboratory 19 months	50	40.0
In soil in closed mason jars 36 months	150	0.0
In soil out of cultivation 36 months	200	1.5
In soil cultivated to dry-land crops for 36 months	95	6.3

remained viable in air-dried rice soil for 6½ mo., in moist rice soil for 4½ mo., in water for 10½ mo., but not for 12½ mo., and under dry conditions in vitro for 17½ mo. Nisikado and Hirata[5] reported that the sclerotia remained viable in an air-dry incubator for periods of 3 yr. at 20° C., 10 to 13 mo. at 25° to 30° C., and 4 mo. at 35° C.; submerged in tap water at 5° C. for 3 yr., at 20° C. for 2 yr. and at 30° C. for 1 yr.

CONCLUSIONS

The rice stem-rot fungus, *Leptosphaeria salvinii,* can remain viable for at least 6 years in uncultivated rice soil. After 2 years viable sclerotia were recovered from stubble in the field and from stubble stored in the laboratory under less variable conditions after about 2½ years. Rotation periods of 4 to 6 years apparently will not eliminate the disease.

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EPINASTY OF TOMATO, ONE OF THE EARLIEST SYMPTOMS OF FUSARIUM WILT

FREDERICK L. WELLMAN

(Accepted for publication September 20, 1940)

The classic symptoms of typical tomato Fusarium wilt (*Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R.) are: stunting, leaf yellowing, wilting, and withering, with darkened vascular system in stems and leaves starting at the base of the plant and progressing upwards until it dies. In connection with certain phases of Fusarium attack, the writer has studied some early symptoms of infection that are not usually mentioned. It is believed that epinasty as one of the symptoms of this disease has never been reported and that it is of special interest.

Observations made 3 times daily on large populations of healthy and inoculated tomato plants, grown under previously described conditions,[1] showed that epinastic response (Fig. 1) is a primary Fusarium-induced disease effect. The response is shown in characteristic fashion by cotyledonary, juvenile, and ordinary leaves and may cause slight inclination of the main stem.

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[5] Nisikado, Y., and K. Hirata. Studies on the longevity of sclerotia of certain fungi under controlled environmental factors. Ber. Ohara Inst. 7: 535–547. 1937.

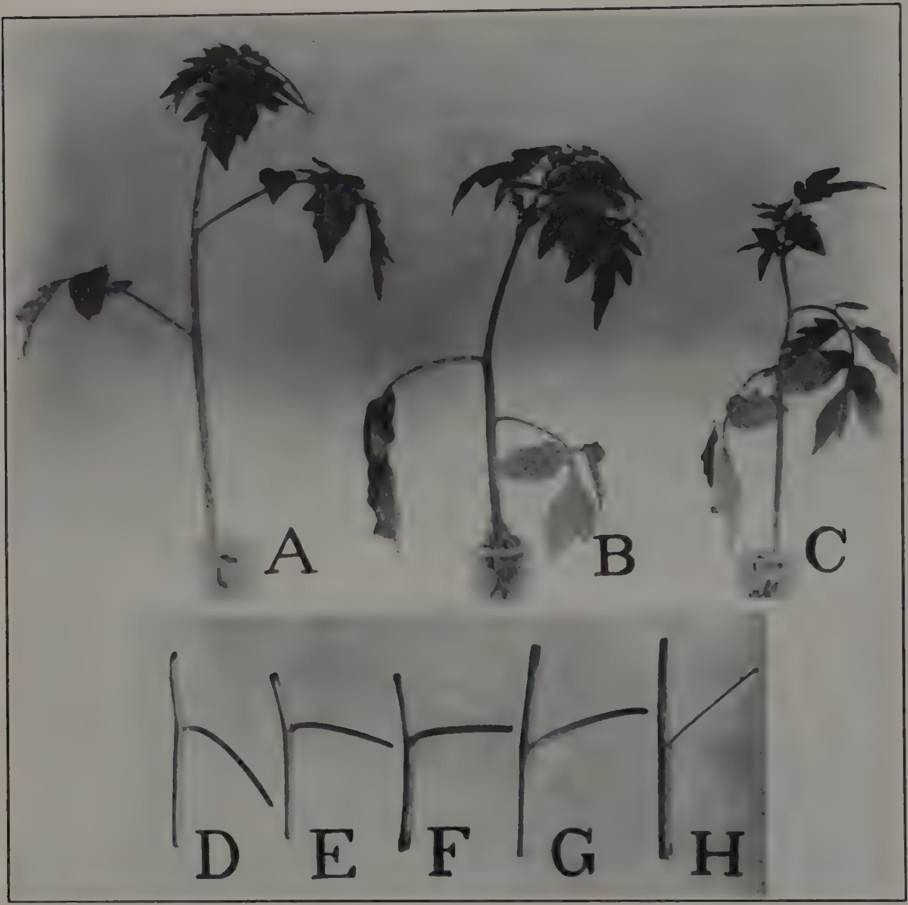

FIG. 1. Epinasty of tomato due to infection by the wilt *Fusarium*. A. Healthy plant. B. Symptoms 5 days after inoculation: epinastic but turgid juvenile leaf; next leaf wilted, attached to epinastic petiole; curvature of upper leaves due to epinasty. C. Epinasty in plant 3 days after inoculation; no wilting. D, E, F, G. Degrees of epinasty of petioles of first leaf above the juvenile 5 days after inoculation (compare with healthy H).

In certain seedlings a single lateral root was inoculated and infection and invasion were evidenced by leaf epinasty on one side of the plant. Pure cultures of *Fusarium* were repeatedly isolated from leaf traces leading to such epinastic leaves, while similar bits of vascular tissue connected with leaves of normal appearance on the same plants were sterile. Epinasty may be easily overlooked since this deflection or droop may be readily interpreted as first wilting. Cook[2] mentioned that infected plants have "intensely dark green leaves flaccid and slightly drooping but not wilted." When tested by lifting with the finger, petioles of epinastic leaves are turgid and held rather stiffly, and are oriented in a characteristic downward-bent position. Petioles of healthy leaves are turgid but resilient and more upright. Measurements were made of angles formed between stem and the first mature-leaf petiole on 18 epinastic and 10 healthy plants. Angles on diseased plants varied between 67° and 136° from the vertical, and on healthy plants between 51° and 55°. Examples illustrating such angles are D, E, F, G, and H in figure 1.

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[1] Wellman, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopath. 29: 945–956. 1939.

Notes were kept on the sequence of disease effects in several series of Bonny Best plants under standard conditions.[3] The following course of symptoms taken from observations on one plant is about typical: 1st day, plant 5 in. high when inoculated, 5-leaf stage; 3rd day, cotyledons and juvenile leaf epinastic, plant otherwise normal; 4th day, cotyledons chlorotic and dislodged, juvenile leaf yellowing, 2 leaves above juvenile showed epinasty, veins in base of plant darkened from infection, could be seen through cortex; 5th day, wilt of juvenile and next leaf above, chlorosis and epinasty of 3 remaining leaves and bud, plant 6 in. high, control 7½ in., development of root knobs along stem up to the leaf above the juvenile; 7th day, all leaves wilted and remaining attached, stem upright but turgid, plant 6 in. high, veins dark to within short distance of terminal bud; 9th day, leaves dead but still attached, watery softening of stem, all vascular system darkened, plant prostrate, stem 6 in. long with 5 leaves. Healthy plant on 9th day, 9 in. high, 8 leaves.

Crocker and coworkers[4,5] have described epinasty and root stimulation on tomato due to chemical treatment. Locke, Riker, and Duggar[6] and Link, Wilcox, and Link[7] observed epinasty and root initiation due to a bacterial infection (*Phytomonas tumefaciens*). The writer has observed similar leaf and root effects caused by attacks of the fungus that produces tomato fusarium wilt. In greenhouse material examined by the writer, root initials were found originating in the pericycle, causing subepidermal swellings in the vicinity of infected vascular bundles. Fisher[8] made first mention of such root initials as a tomato-wilt symptom.

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[2] Cook, W. S. Relation of nutrition of tomato to disposition to infectivity and virulence of *Fusarium lycopersici*. Bot. Gaz. 98: 647–669. 1937.

[3] Wellman, F. L. See footnote 1.

[4] Crocker, W., P. W. Zimmerman, and A. E. Hitchcock. Ethylene-induced epinasty of leaves and the relation of gravity to it. Boyce Thomp. Inst. 4: 177–218. 1932.

[5] Zimmerman, P. W., W. Crocker, and A. E. Hitchcock. Initiation and stimulation of roots from exposure of plants to carbon monoxide gas. Boyce Thomp. Inst. 5: 1–17. 1933.

[6] Locke, S. B., A. J. Riker, and B. M. Duggar. A growth hormone in the development of crown gall. Phytopath. 27: 134. 1937.

[7] Link, G. K. K., H. W. Wilcox, and A. DeS. Link. Responses of bean and tomato to *Phytomonas tumefaciens*, *P. tumefaciens* extracts, B-indoleacetic acid, and wounding. Bot. Gaz. 98: 816–867. 1937.

[8] Fisher, P. L. Physiological studies on the pathogenicity of *Fusarium lycopersici* Sacc. for the tomato plant. Maryland Agr. Exp. Stat. Bull. 374. 1935.

PHYTOPATHOLOGICAL NOTES

A Technique for Identifying the Loose Smuts of Barley.—Loose smut in barley in the United States is caused by *Ustilago nuda* (Jens.) Kell. and Sw. or *U. nigra* Tapke. Not infrequently, both occur in the same fields. The smutted heads and chlamydospores produced by the two smuts are so much alike that positive identification by observation of these characters alone usually is difficult. A definite knowledge of the smut species at hand is necessary, however, if one is to make sound recommendations for control through seed treatment. *U. nuda* requires the difficult hot-water method, which few growers care to use, while *U. nigra* is readily controllable by simple seed dusting with a chemical fungicide. A method of identification based on spore germination has been developed that gives quick and satisfactory results.

Two to three cu. cm. of melted 2 per cent potato-dextrose agar are poured into a 50-mm. Petri dish. While it is hardening, a sterile transfer needle is thoroughly prodded into and among the loose smut heads in the sample and into the spore dust that usually accumulates on the bottom of the container. The needle is then dipped and shaken in about 2 cc. of sterile water in a test tube. This is repeated several times until a representative sample of spores has been put into the water and the latter shows a faint tint of yellow from the spores in suspension. After thorough shaking, the spore-suspension is poured over the surface of the medium in the Petri dish and allowed to stand a few moments until the spores settle to the agar. The water is then poured off and the base of the Petri dish is inverted and pressed down firmly on a clean blotter or paper towel to remove all free water. Moisture that may have collected on the inside of the cover of the Petri dish while the agar was cooling also should be flamed away, and the cover replaced. With these precautions the spores will stick in place on the agar and not float about in excess water when ready for observation. The spores should then be left to germinate at 65–70° F. for 12–18 hours. The cultures are then usually ready for examination. Under these conditions, the spores of *Ustilago nuda* germinate by producing only branching mycelial threads (Fig. 1, A), while the spores of *U. nigra* produce a short promycelium whence four lateral sporidia normally develop. The sporidia multiply rapidly by yeast-like budding (Fig. 1, B). When both *U. nuda* and *U. nigra* occur in the same collection, then, obviously, both the mycelial and sporidial types of spore-germination will take place (Fig. 1, C). It is evident from figure 1 that with this technique an excellent distribution of spores over the surface of the agar is obtained. It is thus possible to observe the behavior of each individual spore and to determine precisely whether the collection is *U. nuda, U. nigra,* or a mixture of these.

The spores of *Ustilago nuda* are usually relatively short-lived. It is recommended that specimens held for later identification be stored immedi-

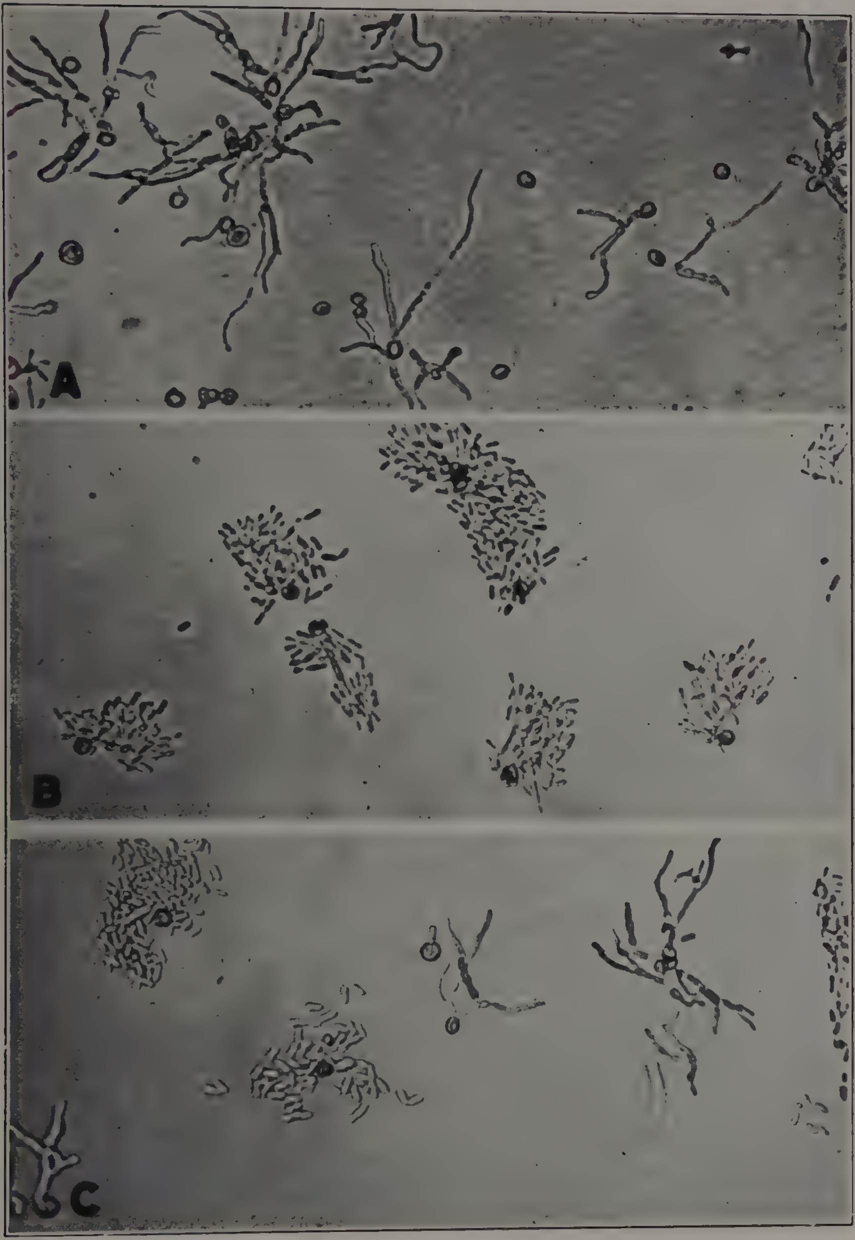

FIG. 1. A. The mycelium-producing spores of *Ustilago nuda*. B. The sporidia-producing spores of *U. nigra*. C. Spore germination in a mixed collection of *U. nuda* and *U. nigra*. ×275.

ately at 34–38° F. The duration of spore viability in both *U. nuda* and *U. nigra* is appreciably prolonged at these temperatures.

It may be noted in conclusion that spores of the barley covered smut (*Ustilago hordei*) may be readily distinguished from those of the two loose smuts. The former are perfectly smooth, the latter echinulate.—V. F. TAPKE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

A Copper-soap Spray for Control of Tobacco Downy Mildew.—A home-made copper-soap spray has effectively controlled downy mildew or blue mold (*Peronospora tabacina* Adam) of tobacco. This spray, called Flordo, is cheaply and easily made from copper sulphate ($CuSO_4$) and soap, as described by Shippy.[1] A spray consisting of 1 lb. $CuSO_4$, and 5 lb. of soap to 50 gal. of water was found by Clayton *et al.*[2] to give excellent control in some instances, but was inconsistently effective. A spray made with $CuSO_4$ and potash soft soap was recommended by Mandelson[3] for use in Queensland, Australia.

Flordo is readily prepared by dissolving the copper sulphate and soap separately, each in half the required amount of water in barrels or drums, and dumping the copper sulphate solution into the soap solution. Other methods of mixing are described by Shippy. When properly made, the spray is free of sticky curd. If, however, a small amount of curd is formed, it may be removed by straining. The spray keeps for weeks, and any sediment formed can be stirred back into suspension. During ordinary use, no serious corrosion of iron vessels, either galvanized or plain, has been noticed. The stability of Flordo is of particular advantage in spraying small beds, since enough spray can be made at one mixing to serve for several applications or perhaps for the entire spraying season.

The excellent wetting, spreading, and adhesive properties of Flordo make it very satisfactory for spraying tobacco. A visible coating of residue remains on the leaves for several days after application, even during rainy weather.

The concentration of Flordo first used in experiments on tobacco had a metallic copper content approximately equal to that of a cuprous oxide-oil emulsion spray, containing 1 lb. of cuprous oxide (Cu_2O) per 100 gal., with which Flordo was compared. For the purpose, 3.5 lb. of $CuSO_4 \cdot 5H_2O$ and 14 lb. of high grade oleate soap were used per 100 gal. Other concentrations tried were $\frac{1}{2}$ and $\frac{1}{4}$ of the above; for convenience these will be referred to as half-Flordo and quarter-Flordo, respectively. The proper ratio of soap to $CuSO_4$ varies somewhat with the type of soap and the hardness of the water.

Flordo has been tried for control of downy mildew in Florida on flue-cured tobacco plant beds, cigar-wrapper tobacco plant beds, and cigar-wrapper tobacco in the field. The plants were sprayed with Flordo about twice a week, in direct comparison with cuprous oxide-oil emulsion spray.

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[1] Shippy, W. B. Flordo spray. Fla. Agr. Exp. Stat. Press Bulls. 504 (1937) and 547 (1940).

[2] Clayton, E. E., J. G. Gaines, T. E. Smith, W. M. Lunn, and K. J. Shaw. Control of the blue mold (downy mildew) disease of tobacco by spraying. U.S.D.A. Tech. Bull. 650: 22 pp. 1938.

[3] Mandelson, L. F. Additional recommendations for the control of blue mold of tobacco. Queensland Agr. Jour., Dec., 1933.

Full-strength Flordo was used on a flue-cured bed in Madison County in 1939, and the downy-mildew control obtained possibly was a little better than that with the cuprous oxide-oil emulsion spray. A similar comparison was made with half-Flordo, in 1940, with equally good results. No injury to the plants, either in the plant bed or in the field, was observed.

On cigar-wrapper beds in Gadsden County, in 1939, both full-strength Flordo and half-Flordo gave good control of downy mildew but caused con-

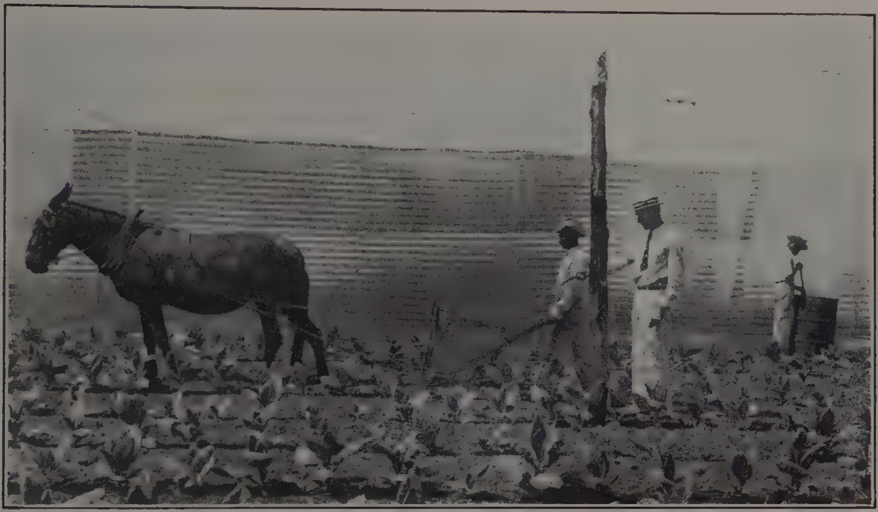

FIG. 1. Spraying cigar-wrapper tobacco in the field for downy-mildew control.

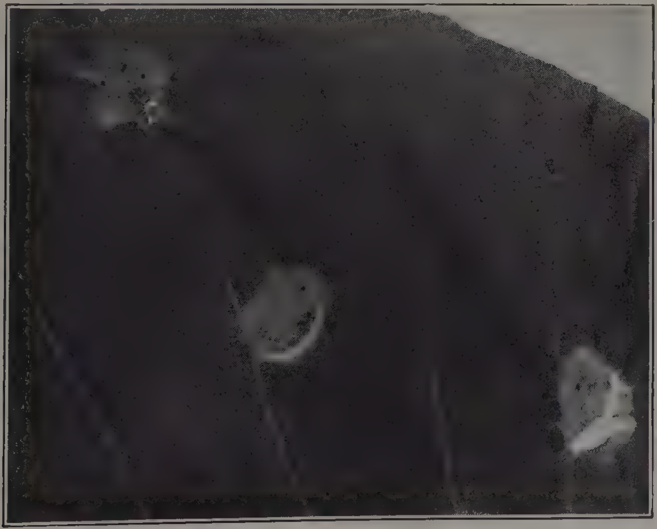

FIG. 2. Downy-mildew spots on a cigar-wrapper tobacco leaf.

siderable stunting of the plants.[4] Tests made in 1940 under conditions not very favorable to the disease indicated that quarter-Flordo with 1 per cent cottonseed oil may effectively control without stunting.

Cigar-wrapper tobacco, transplanted to the field April 10, 1940, was sprayed (Fig. 1) five times at 3-day intervals, beginning May 4 and ending May 16, using about 50 gal. of half-Flordo per acre per application. An outbreak of downy mildew (Fig. 2) occurred from May 12 to May 14 on nonsprayed checks, while almost no signs of the disease appeared on the sprayed plants. Spray residue was noticeable on the leaves on May 25 after a rain of 1.14 inches, but not on May 30 after another rain of 1.64 inches. These observations indicate that cigar-wrapper tobacco in the field in Florida may be satisfactorily sprayed with Flordo for downy-mildew control until about mid-May, which covers the period when serious outbreaks of the disease generally occur.

Possible explanations of the effectiveness of Flordo for downy-mildew control on tobacco may lie (1) in a direct fungicidal action of the copper-soap coating on the leaves, and (2) in increasing the wettability of the leaves, which permits water to spread into a thin, quickly drying film on sprayed leaves, in contrast to the formation of nearly spherical, slowly drying drops on unsprayed leaves. This characteristic of sprayed leaves has been observed to reduce strikingly the period of time during which the leaves remain wet with rain or dew, and would logically reduce the chance for infection with downy mildew and possibly other leaf diseases.—RANDALL R. KINCAID, North Florida Experiment Station, Quincy, Florida.

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[4] Mandelson (*loc. cit.*) reported that too much soap tended to cause spray injury. The possibility of eliminating stunting of cigar-wrapper tobacco plants in Florida by reducing the amount of soap to a minimum has not yet been fully investigated.

HAVEN METCALF

HAVEN METCALF
(1875–1940)

E. P. MEINECKE

Plant Pathology has suffered a heavy loss. Dr. Haven Metcalf found his death after years of great distress on May 23, 1940, at Washington, D. C.

Haven Metcalf was born at Winthrop, Maine, on August 6, 1875, of old New England stock. His family, of sturdy English yeomanry, as he himself described it, established itself in this country in 1637. It looks back upon a distinguished history during which it furnished its large share to the learned professions from the days of the Colonies to the present day. The spirit of adventure drove his father, as a young man, to California in the early days of the gold rush, but not for long. He soon returned and finally settled on the Maine farm which became the home of young Haven. At Winthrop, Metcalf received his first schooling. After passing through high school he went to Colby College from 1892 to 1894 and then shifted to Brown, where he received the degrees of A.B. in 1896 and A.M. in 1897. Metcalf continued his studies at Harvard in 1899, at Indiana in 1900, and finally at Nebraska, where he received the degree of Ph.D. in 1903.

His first experience with teaching came to him at Brown, where he taught botany as an instructor from 1896 to 1899. From 1899 to 1901 he was professor of biology at Tabor College. At Nebraska he was an instructor in bacteriology from 1901 to 1902. In 1902 he accepted his first important position as associate professor in botany and bacteriology at Clemson Agricultural College, S. C., and State Botanist of South Carolina, both of which positions he held until 1906. During this period he lectured at Martha's Vineyard Summer Institute from 1896 to 1899 and at the South Carolina State Summer School from 1903 to 1905. In 1904 and 1905 he was Special Agent in the U. S. Department of Agriculture. The year 1906 brought the decisive turning point in his career when he was appointed to the position of Pathologist in the Bureau of Plant Industry in the Department of Agriculture. In 1907 he organized and became Pathologist in charge of the Laboratory of Forest Pathology of that Bureau. This position was followed in 1924 by that of Senior Pathologist in charge, Office of Forest Pathology, and, in 1928, by that of Principal Pathologist in charge of the Division of Forest Pathology of which he was the head until his death.

Two events that had a strong influence upon his development occurred during this period. In 1908 he visited Italy and in 1923 he represented the United States Government at the International Conference on Phytopathology in Holland. In the United States, recognition came to him in many forms. He was a member of Sigma Xi, Phi Beta Kappa, and Delta Upsilon. In 1913 he served as Vice President and in 1914 as President of the American Phytopathological Society and from 1911 to 1913 as an associate editor of Phytopathology. The Botanical Society of Washington elected him President in 1919. In the years 1925 and 1926 he was President of the National Shade Tree Conference. He held memberships in the American Association for the Advancement of Science (fellow), the American Botanical Society, the American Phytopathological Society (life member), the Botanical Society of Washington, the National Shade Tree Conference, the New England Botanical Club, the Society of American Bacteriologists, the Society of American Foresters, the Royal Scottish Arboricultural Society, the Washington Academy of Sciences, and the Washington Biological Society.

On the whole, Haven Metcalf's life was not an easy one. Ill health followed him throughout its course, though in middle age he enjoyed a modicum of well-being and even robustness. In the diary of the old family physician he found an entrance of his birth with the remark: "Child will not live." All through his younger days he was handicapped by ill health and financial troubles. His childhood was a lonely one. The last child and the only boy in the family, he was separated by ten years from the youngest sister. Few of his early aspirations and interests found nourishment in the soil of New England farm life, though sympathy and understanding for the gifts of the boy were not lacking. His early leanings were toward medicine, a profession for which he would have been eminently fitted. That his family could not afford to let him follow this dream threw a shadow over his young life. After high school he left the farm and threw himself with all his energy into the world of learning where he felt more at home. The quiet and intimate atmosphere of the small college he attended influenced him profoundly. It was here where he acquired a fund of factual knowledge and received the training in disciplined thought that so marked his later life. His years at Brown, beginning with premedical courses, gave him the final orientation towards botany, bacteriology and mycology. Towards the end of his days at Brown he married Flora May Holt, with whom he had studied at Colby and Brown. Herself a highly gifted woman of old New England ancestry, she shared with him his deep and keen interests in music, art and literature. Her tragic death, after thirty-six years of the happiest married life, threw dark shadows over his last years.

It was at the University of Nebraska where Haven Metcalf came under the influence of Professor Charles E. Bessey, that excellent botanist and remarkable educator. The imprint of Bessey's outstanding personality remained with him throughout his life. Under Bessey's wise and firm guidance, Haven Metcalf acquired that scientific integrity and honesty with himself which became so prominent a feature of his character and which he equally and uncompromisingly demanded of others. Metcalf's first appointment to a responsible position at Clemson College not only gave him further experience in teaching but it introduced him to the South, to Southern life and attitude towards life. It fascinated him, and throughout later years he would speak of those years with deep understanding and a certain nostalgia. During this time he had his first contacts with the Federal Government, which finally led to his appointment in the Bureau of Plant Industry and the definite establishment in his life work.

At that time the field of forest pathology was not represented at Washington. Forest-tree diseases had been a subject of study at the short-lived Mississippi Valley Laboratory, (U. S. Department of Agriculture) located at the Missouri Botanical Garden, St. Louis, Mo., where Dr. Hermann von Schrenk, Dr. George G. Hedgcock, and Dr. Perley Spaulding had done valuable pioneer work for several years. To Haven Metcalf, then entering his thirties, the transfer to Washington with its promise of close contacts with a number of distinguished scholars and its opportunities for creative work on a large scale was the first step into a new and wide world. But it cannot be denied that the general attitude towards forest pathology as a new and vital discipline was by no means propitious. In 1906 phytopathology, as a whole, still had to fight for its existence, in spite of the brilliant achievements it had to its credit. Where it obviously could show immediate practical results, as in the study and control of short-rotation crops, it was tolerated as a legitimate if not quite respectable offshoot of mycology, itself not always regarded as genuinely botanical in nature. Farlow and Thaxter had raised American mycology to the first rank in international science. Neither of the two had any interest in applied mycology. Furthermore, the application of plant pathology to crops, which did not mature for many decades or for centuries, seemed a futile undertaking; and, even in high places, the opinion was widely spread that the study of forest-tree diseases had more of an academic than of a practical character. Robert Hartig, the creator of modern forest pathology, had the same experience. At the time when he published his classical studies on the diseases of the forest, mycology stood at the zenith of its development in Europe. General plant pathology was hard put to it to establish itself as a legitimate branch of learning. The pathology of the forest tree was still a neglected field. For his concept of the pathology of the forest community as a whole Robert Hartig found little understanding, even among professional foresters, until later in life.

When Haven Metcalf was put in charge of investigations in forest pathology Robert Hartig's work was none too well known in the United States outside of its mycological aspects. Haven Metcalf early recognized the great practical possibilities of systematic research in a field as rich as ours. Here were immense virgin forests, wild and uncared for, representing huge potential wealth so long as the loss caused by decay and parasitic fungi could be held in reasonable check. With his characteristic energy and planning ability, Metcalf went to work. From small beginnings he built up an efficiently working organization, which at no time became top-heavy. He never yielded to the temptation to seek expansion for expansion's sake.

Almost at once he was confronted with a problem of the first magnitude.

When the chestnut blight first made its appearance in the United States, few men realized its extremely serious nature. Leading mycologists and pathologists were inclined to consider the causative fungus as native to this country. Unfavorable weather conditions had, so it was thought, given the fungus a temporary advantage over the weakened trees. A change in weather conditions to the better would soon restore the balance. The diagnosis was further complicated by the fact that the fungus consistently found in connection with the chestnut blight was so closely related to a harmless native fungus on chestnut that the two could only with difficulty be distinguished from each other. Furthermore, in all the epidemiological experience of the times, there was no precedent that could serve for a rational approach to the problem of dealing with the new and ominous disease.

Haven Metcalf was convinced that the fungus must have been introduced from foreign countries, most probably from eastern Asia. The careful work of C. L. Shear and his collaborators finally established the correctness of this view; but by the time it was universally accepted, the disease had made such devastating progress that a program of control had but little chance of success. Metcalf devoted not less than 19 publications to the chestnut blight. He fought a hard fight against the apathy of professional mycologists and pathologists and against that of the general public which could not realize that such a thing as a fungus could actually destroy its fine old chestnut stands. There existed no machinery ready to go into action. No funds were available except for a more or less academic study of the disease. When the rapid and disastrous spread of the blight made it clear that the American chestnuts were doomed to extinction Metcalf devoted himself to a vast project of attempting to replace them, at least in part, with carefully selected immune or resistant species from Eastern Asia.

From the early years of his concern with the chestnut blight dates one of Haven Metcalf's most fruitful contributions to plant pathology. He was among the first to recognize and to stress the need for a well-planned and strictly enforced system of quarantines applied to the movement of tree nursery stock within the country and from foreign countries into the United States. The Federal Government was powerless to act within the States, and public indifference prevented that quick and sustained action, which was so urgently needed. Metcalf never gave up the fight against what he termed "free trade in plant diseases and insect pests."

How sound his reasoning was became evident when a few years later a second disaster struck the American forests. The five-needle pines, the valuable white pine of the East and later the related species of the Far West were attacked by the white pine blister rust. The disease had been imported on nursery stock from Germany and France. Had there existed an effective quarantine service by the beginning of the century, the country would in all probability have been spared not only incalculable loss but also the ever recurrent cost of control. The experience gained in the chestnut blight campaign stood Metcalf in good stead when the blister rust began to make heavy inroads in the forests. Both the strategy and the tactics of the fight against the disease testify to his clear thinking and his talent for organization. What has been accomplished under his guidance stands forever to his credit. The same qualities showed themselves again in his handling of the larch canker, a confusing canker of Douglas fir, and the Dutch elm disease. No matter what the problem, he would invariably bring to it intellectual honesty, clean research, and a sense of the realities. In earlier years he was much interested in rice diseases. From Italy, where he had gone to continue his research in this field, he brought back a variety which is widely cultivated in the Sacramento Valley in California under the name of Colusa rice. This practical sense accounts for his strong interest in the protection of shade trees from disease. In the last decades, a large industry has sprung up which concerns itself with the care of shade and ornamental trees. His restraining influence, flowing from his insistence upon rigid research, has done much to prevent the new branch of so-called tree surgery and tree medication from falling into disrepute.

Forest pathology was infinitely more to him than a detached and academic study of a disease complex. That in every case the immediate and remote causes must be investigated systematically and in detail was self-understood. But never did he lose sight of the fact that etiological knowledge alone is of little help unless it is paired with a clear understanding of tree physiology and of the ecological factors that govern the life of the forest community. To him a disease represented an additional ecological element in the forest. Beyond the effect on the individual tree he visualized its bearing on the present and future development of the forest, and he was among the first in the United States who realized that forest pathology in the Hartigian sense must ever go hand in hand with silviculture, which is the practical application of tree physiology and forest ecology.

With the years Haven Metcalf grew to a figure of real stature. One could not meet him without sensing his inner strength. Independence of thought, breadth of outlook and a highly developed faculty of diagnosis, probing down through the surface to the essential kernel of a problem, were his strongest characteristics.

Personally he had great charm. A man of poise, courteous and friendly, a master of concise and finely chiselled language, he was an outstanding figure in any group of men. His deep interest in art, in music, in philosophy and literature, coupled with striking originality of thought and expression, made his conversation an experience to be remembered. With his wisdom and inborn kindliness, he had an extraordinary influence on younger men whom he was ever ready to help in word and, just as often, in deed. Severe with himself, he was intolerant of pompous mediocrity and equally intolerant of shallowness and undisciplined thinking. But he was quick to recognize promise and worth in others, and once he had given his sympathy he held to it with unswerving loyalty.

It was an honor to hold his respect. It was a greater honor to have his friendship.

LIST OF PUBLISHED PAPERS

1 The collection and preservation of fungi. *In* ''Botanizing,'' by Bailey, W. W. (Preston and Rounds, Providence, R. I.) 1899.

2 The foundations of agricultural teaching. U. S. Dept. Agr. (O. E. S.) Bull. 142: 170–172. 1904. (Proc. 17th Ann. Con. Assoc. Am. Agr. Col. and Exp. Stat. 1903).

3 Certain problems relating to the individuality of chromosomes. Proc. Neb. Acad. Sci. 7: 109–111. 1901.

4 Notes on the ferns of Maranocook, Maine. Rhod. 3: 236–237. 1901.

5 Review of Gorham's bacteriology. Science (n.s.) 15: 188–189. 1902.

6 New apparatus for phytopathological work (With G. G. Hedgcock). Jour Appl. Mic. and Lab. Meth. 6: 2494–2495. 1903.

7 Eine durch Bakterien verursachte Zuckerrübenkrankheit. Zeitschr. Pflanzenkr. (With G. G. Hedgcock). 12 (1902): 321–324. 1903.

8 Cultural studies of a nematode associated with plant decay. Trans. Am. Mic. Soc. 24: 89–102. 1903.

9 Cultural studies of a nematode. Science (n.s.) 18: 728. 1903.

10 A soft rot of the sugar beet (*Bacterium teutlium* Metcalf). Ann. Rpt. Neb. Agr. Exp. Stat. 17: 69–112. 1904.

11 *Bacterium teutlium*, sp. nov. Cent. f. Bakt. 13: 28–30. 1904.

12 Sanitary conditions in the home and on the farm. S. C. Agr. Exp. Stat. Bull. 89. 1904.

13 The new fungous disease in the South Carolina rice fields. Rice Indus. 1904.

14 Report of the botanist and bacteriologist. (16th Ann. Rpt. S. C. Agr. Exp. Stat.) (1903): 13–16. 1904.

15 Clemson College Science Club. Science (n.s.) 20: 537, 764–765. 1904; 21: 35, 788–789, 22: 248–249. 1905.

16 The present status of the chestnut bark disease (With J. F. Collins). U. S. Dept. Agr. Bull. 141 (part 5): 45–54. 1905.

17 Organisms on the surface of grain, with special reference to *Bacillus coli*. Science (n.s.) 22: 231. 1906.

18 Sanitary conditions in South Carolina. Science (n.s.) 23: 231. 1906.

19 A preliminary report on the blast of rice, with notes on other rice diseases. S. C. Agr. Exp. Stat. Bull. 121. 1906.

20 Farm, home, and school sanitation. Clemson Agr. Coll. Exten. Work. 3: 1–32. 1907.

21 The scientific contributions of Henry Bennett Slade. Brown Alumni Monthly 7: 139–140. 1907.

22 The pathology of the rice plant. Science (n.s.) 25: 264–265. 1907.

23 Review of Peglion's Le mallatie crittogamiche delle piante coltivate. Science (n.s.) 25: 273–274. 1907.

24 Bark disease of the chestnut. The Nut Grower 6: 1–3, 5. 1908.

25 The immunity of the Japanese chestnut to the bark disease. U. S. Dept. Agr. Bull. 121. 1908.

26 The bark disease of the chestnut. American Fruit 8: 20. 1908.

27 Diseases of ornamental trees. U. S. Dept. Agr. Yearbook (1907): 483–494. 1908.

28 The Botanical Society of Washington. Science (n.s.) 27: 586, 820–821 and 28: 816. 1908.

29 A proposito del brusone del riso. (Introduction by R. Farneti) L'Alba Agric. 7: 1414–1415. 1909.

30 Farneti, Rodolfo. A proposito del brusone del riso. L'Alba Agric. 7 (Abstract in Riv. Patol. Veg. (n.s.) 3: 276–277. 1909).

31 Plant diseases in 1908; forest and shade trees. U. S. Dept. Agr. Yearbook (1908): 537–538. 1909.

32 The present status of rice blast. Science (n.s.) 29: 911. 1909.

33 The present status of the bark disease of the chestnut. Science (n.s.) 31: 239. 1910.

34 The chestnut bark disease (With J. F. Collins). Science (n.s.) 31: 748. 1910.

35 Further notes on the bark disease of the chestnut. (With J. F. Collins). Phytopath. 1: 106. 1911.

36 The chestnut blight. Country Gentleman, Aug. 3, 1911.

37 The control of the chestnut bark disease (With J. F. Collins). U. S. Dept. Agr. Farmers' Bull. 467. 1911.

38 Editorial on Chestnut Blight Congress. Phytopath. 2: 91. 1912.

39 Forest fungi suppression. Boston Transcript. Feb. 12, 1912.

40 Fungous diseases of the chestnut and other trees. Boston Transcript. 1912.

41 The chestnut bark disease. Jour. Econ. Ent. **5**: 222–226. 1912.

42 The chestnut bark disease. U. S. Dept. Agr. Yearbook (**1912**): 363–372. 1913.

43 The present known distribution of the chestnut bark disease (With J. F. Collins). Science (n.s.) **35**: 420–421. 1912.

44 Chestnut tree blight (*et al.*). Senate Doc. **653** (62nd Congress): 1–36. 1912.

45 Diseases of the chestnut and other trees. Trans. Mass. Hort. Soc. (**1912**): 69–95.

46 Diseases of rice (In *The Diseases of Tropical Plants* by Mel T. Cook (Macmillan Co. New York) 99–106. 1913).

47 Discussion of J. W. Toumey's paper: "What should be done with the chestnut stands in Southern New England?" Proc. Amer. For. **9**: 43–45. 1914.

48 The chestnut bark disease. Jour. Hered. **5**: 8–18. 1914.

49 Two eastern forest diseases which threaten the Pacific States. Phytopath. **5**: 291. 1915. (Science (abstract) **42**: 580. 1915.)

50 Spread of the chestnut blight in Pennsylvania. Phytopath. **6**: 302. 1916.

51 The pine blister disease problem as a whole. Amer. For. **23**: 72–74. 1917.

52 The chestnut blight disease. Rpt. Proc. Northern Nut Growers' Assn. **7**: 41–47. 1917.

53 The control of the white pine blister rust. Jour. Wash. Acad. Sciences (Abstract) **7**: 313–314. 1917.

54 Summary of the white pine blister rust situation "White Pine Blister Rust": pub. by Comm. on the suppression of the white pine blister in N. America. 1918.

55 Summary of the white pine blister rust situation. Jour. Forest **16**: 85–89. 1918.

56 The problem of the imported plant disease as illustrated by the white pine blister rust. Brooklyn Bot. Gard. Mem. **1**: 327–333. 1918.

57 Summary of the white pine blister rust situation. Rpt. on White Pine Blister Rust Control (**1918**): Bull. 2, p. 16. (Amer. Plant Pest Comm. (Boston)). 1919.

58 The story of a plant introduction. Jour. Wash. Acad. Sciences **11**: 474. 1921.

59 White pine blister rust in the Northwest. Phytopath. **13**: 46. 1923.

60 Chestnut blight (*Endothia parasitica* (Murr.) A. and A.) in Europe. Phytopath. (Abstract) **14**: 52. 1924.

61 Observations on the Douglas fir canker (*Phomopsis pseudotsugae* Wilson) in Great Britain. Phytopath. (Abstract) **14**: 52. 1924.

62 Less serious diseases of white pine. Bull. Greens Sect. U. S. Golf Assoc. **4**: 147–148. 1924.

63 The red currant question. Rpt. Proc. and Recommend. 9th Ann. Blist. Rust Conf. (**1924**): 107–112. 1924. (Mimeographed).

64 Certain research activities of the Office of Forest Pathology. Ann. Blist. Rust Conf. **10**: 67–69. 1925. (Mimeographed).

65 Investigative work in the Office of Forest Pathology in 1925. Rpt. Proc. Blist. Rust Conf. **11** (**1925**): 81–92. [1926?] (Mimeographed).

66 Flyers and sappers of the enemy. Summ. Proc. 2nd N. E. For. Cong. (**1925**): 13–14. 1925.

67 New gall-rust disease may be menace to pine. Official Record **5**: 1, 2, and 5. 1926.

68 City trees have hard life. Science News Letter (Washington, D. C.) **11**: 225. 1927.

69 Fungous diseases. Papers, For. Protect. Confs. N. Y. State Coll. Forestry (Syracuse) (**1926**): 2–3. 1927.

70 Diseases of park trees and shrubs. Parks: A Manual of Municipal and County Parks **2**: 694–698. 1928.

71 Larch canker, new unwanted immigrant, attacks fir and pine. U. S. Dept. Agr. Yearbook (**1927**): 419–420. 1928.

72 The Dutch elm disease. Proc. 5th Nat. Shade Tree Conf. (**1929**): 60–61. 1929.

73 The European larch canker. Proc. 5th Nat. Shade Tree Conf. (**1929**): 58–59. 1929.

74 The European larch canker (*Dasyscypha willkommii* (Hartig) Rehm.) U. S. Dept. Agr. Pl. Dis. Rptr. Suppl. **75**: 66. 1930.

75 The woodgate rust. Calif. Dept. Agr. Mo. Bull. **19**: 496–497. 1930.

76 Woodgate rust (*Peridermium* sp.). U. S. Dept. Agr. Pl. Dis. Rptr. Suppl. **75**: 68–69. 1930.

77 Dutch elm disease found here. U. S. Dept. Agr. Official Record **9**: 3. 1930.

78 Dutch elm disease found in America. Forest Worker **6**: 12–13. 1930.

79 Science and shade tree welfare. Tenth Nat. Shade Tree Conf. Proc. Ann. Meet. (**1934**): 11–14. 1934.

EXPERIMENTS WITH ERADICANT FUNGICIDES FOR COMBATING APPLE SCAB[1]

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INTRODUCTION

Spraying or dusting with protectant fungicides has long been the dominant measure of control for most fungus diseases of orchard fruit plants. For some years the senior writer and associates have studied potentialities of increased application of the principle of eradication to the control of certain types of these diseases by means of direct chemical attack on the pathogen at a vulnerable stage in its life history. The point of view and the earlier progress of this work have been reported (2, 7, 9) and pertinent literature has been reviewed (7). The research here presented relates to later experiments on apple scab, concerning which preliminary reports have been made (3, 4, 5).[2]

Under the conditions encountered in Wisconsin the only practically important overwintering of the apple-scab pathogen occurs in the fallen leaves, whence comes the ascosporic inoculum that, in the spring, initiates the current season's infection (6). Attack with eradicant fungicides has, therefore, been directed at two potentially vulnerable stages: (a) after harvest and before many leaves have fallen, when the foliage can be thoroughly covered by comparatively drastic sprays designed sharply to limit the production of ascospores; and (b) after leaf-fall, in fall or spring, when still more drastic chemical treatments can be given the dead leaves on the ground without injurious effects on the tree.

ERADICANT FUNGICIDAL TREATMENT OF LEAVES ON THE TREE

The experiments on fall spraying are a continuation of those previously reported (7). By use of the materials and methods developed during these earlier studies it was sought to gain further evidence concerning the effects of eradicant spraying on the epidemiology and control of the disease.

Materials and Methods

Copper sulphate.—Technical copper sulphate crystals.

Elgetol.—A proprietary product containing 19.4 per cent of the sodium salt of dinitro-o-cresol, with a penetrating agent.

Elgetol Extra.—A proprietary product containing 13.4 per cent of the sodium salt of dinitro-o-cresol, with a penetrating agent (in greater amount than in Elgetol).

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Acknowledgments are made to Mr. Donald M. Coe, formerly assistant in plant pathology, for help in performing the experiments.

[2] This work was reported at the Conference on Eradicant Fungicides at the annual meeting of The American Phytopathological Society, Columbus, Ohio, Dec. 29, 1939.

Fish oil.—Cold-pressed menhaden fish oil.

Lime.—Commercial hydrated spray lime containing not less than 99 per cent of $Ca(OH)_2$.

Monocalcium arsenite.—A finely ground commercial product containing approximately 74 per cent total arsenic and 23 per cent water-soluble arsenic (each expressed as As_2O_3).

Zinc arsenite.—A finely ground commercial product containing approximately 41 per cent total arsenic and not more than .5 per cent water-soluble arsenic (each expressed as As_2O_3).

Experimental Orchards and Treatments.—A diagram of the two experimental orchards appears in figure 1. Orchard 1, set in 1930, received the fall sprays. Orchard 2, set in 1931, was nontreated, except that the Yellow Transparent block was given the same treatment as orchard 1 in order to increase the distance between that orchard and the source of ascosporic inoculum in the nonsprayed orchard. Orchard 1 was 300 ft. from the nearest nonsprayed portion of orchard 2.

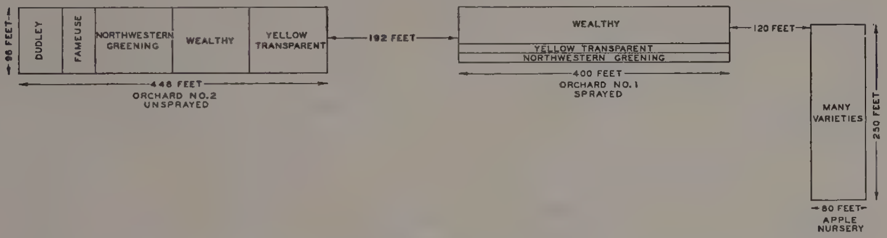

FIG. 1. Map of experimental orchards used in studies of the effects of eradicant spray treatments on the development of apple scab, Madison, Wis., 1936–39.

On Oct. 6, 1936, and Oct. 7, 1937, and 1938, all of orchard 1 was sprayed by means of a power sprayer, the formula used being copper sulphate, 8 lb., monocalcium arsenite, 2 lb., zinc arsenite, 6 lb., fish oil, 3 qt., and water to make 100 gal. Orchard 2 was nonsprayed, except the Yellow Transparent block, which was given the same treatment as orchard 1.

In 1938, ascosporic inoculum from a young apple nursery (Fig. 1) located 120 ft. southeast of orchard 1 blew into the treated orchard, and is thought to have accounted for over half of the infection in this orchard. On April 25–26, 1939, the overwintered leaves on the ground in the nursery and a zone around it were treated with .5 per cent Elgetol Extra to limit the ascosporic inoculum. All leaves were thoroughly wet by the spray.

Epidemiological Records

During the years under consideration, climatic conditions were generally favorable for the production of perithecia. Graphic summaries of pertinent epidemiological data are given in figures 2, 3, and 4.

In 1937, ascospores were ready for discharge by Apr. 29 (Fig. 2). Scab

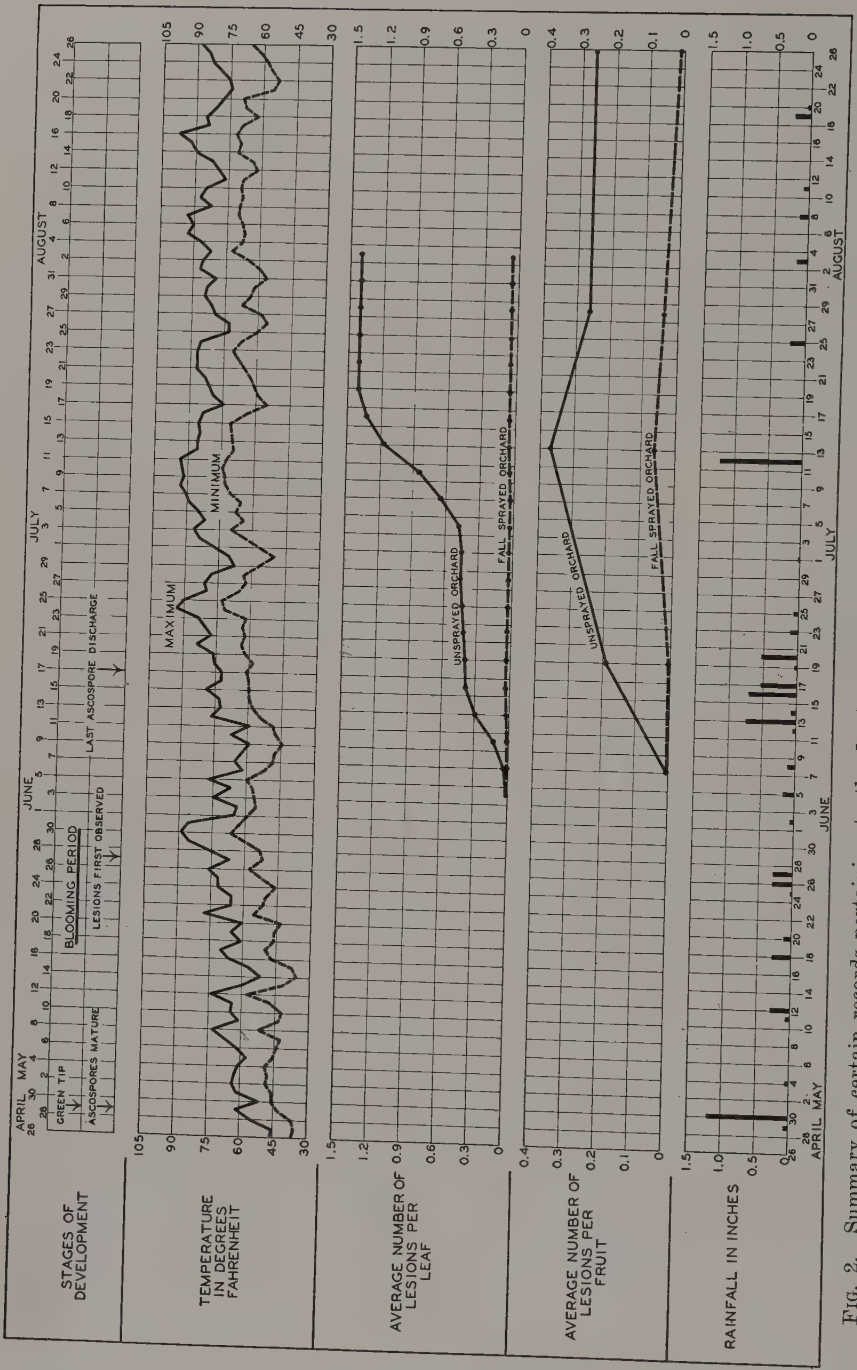

FIG. 2. Summary of certain records pertaining to the development of apple scab on Wealthy in relation to eradicant spraying, Madison, Wis., 1937.

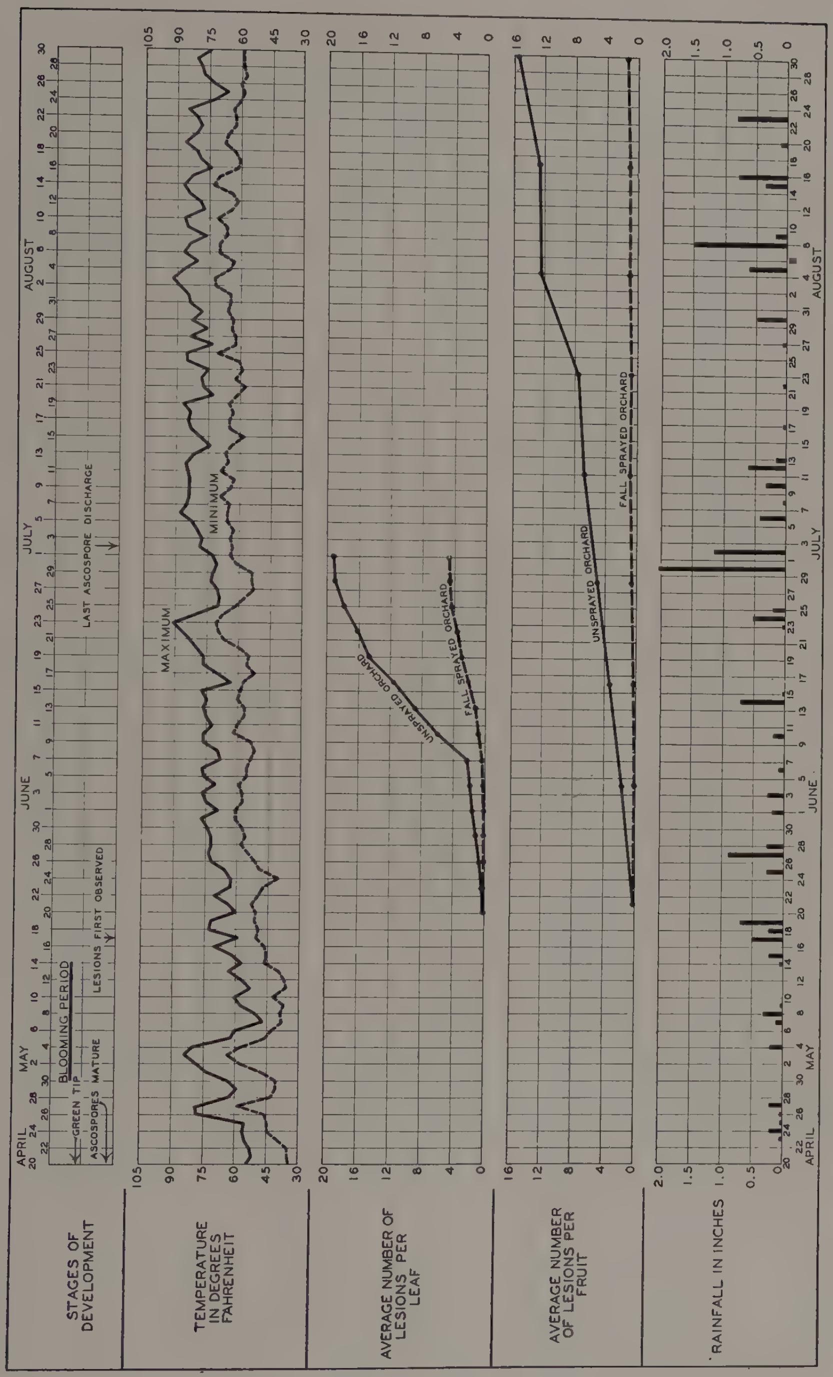

FIG. 3. Summary of certain records pertaining to the development of apple scab on Wealthy in relation to eradicant spraying, Madison, Wis., 1938.

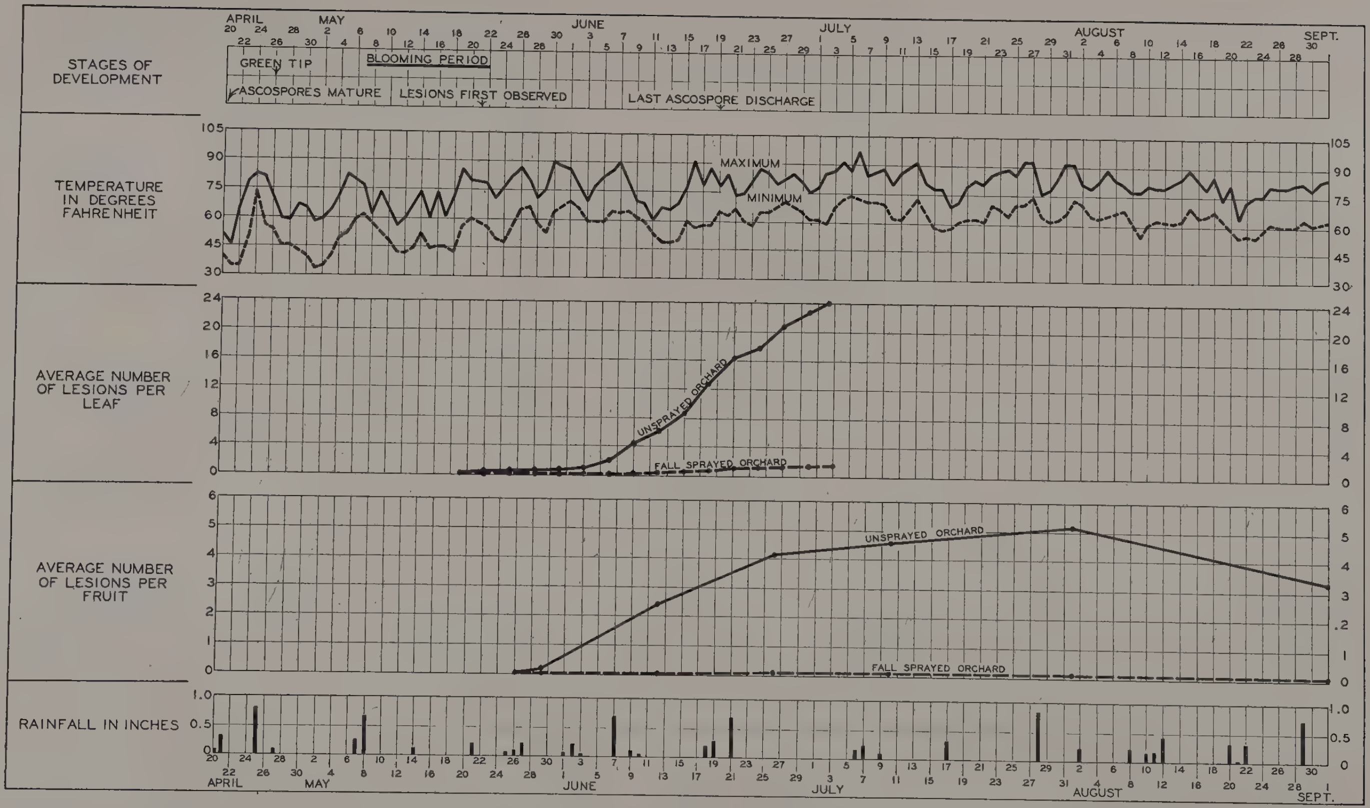

FIG. 4. Summary of certain records pertaining to the development of apple scab on Wealthy in relation to eradicant spraying, Madison, Wis., 1939.

lesions were first observed May 21. The season was unusually dry and scab development was sparse.

In 1938, ascospores matured unusually early, being ready for discharge by March 28. The first infection occurred Apr. 23–25 (Fig. 3). The rainy period of May 15–19 was very favorable for infection. Rains were frequent throughout the season and a severe scab epidemic developed.

In 1939, ascospores were ready for discharge by Apr. 22. The first infection occurred during the rains of May 7–8 (Fig. 4). The first lesions were observed May 21. Wealthy was in full bloom on May 11. The season was dry, especially in the latter half, and not conducive to severe scab development.

Experimental Results

By a method previously described (7) counts were made of the perithecia on 100 leaves collected at random from each, the treated and the nontreated, orchard. The data are shown in table 1. While it is possible that other factors than the spray treatment may have played some part in the differences shown, these results indicate a reduction in incidence of perithecia by the spray in the 3 years in the range of 97 to 100 per cent.

The development of scab until about 2 weeks after petal-fall was followed each year on Wealthy in both the treated and the nontreated orchard by counting at 2- to 4-day intervals the number of lesions on the leaves of 50 spurs taken at random (Table 1). The spur leaves are the earliest exposed, and, being determinately borne, are expanded and become resistant early in the season. The scab reported on these leaves (Table 1) is thought to have resulted from ascosporic infection. The indicated percentage reduction in incidence of lesions on these leaves in the treated orchard as compared with the nontreated in the data of the 3 years ranged from 96 to 100, being in very nearly direct relation to the indicated percentage reduction in perithecia, which ranged from 97 to 100.

Beginning about 10 days after petal fall, when the spur leaves were becoming resistant, the seasonal development of the disease on the foliage was further followed on 20 tagged terminal shoots on as many trees in each orchard. In tagging the shoots equal representation was given to the north, south, east, and west sides of the trees. The number of leaves on each shoot and the lesions on these leaves were counted at 3-day intervals. The results are shown in figures 2, 3, and 4.

The foregoing data were supplemented and checked by large-scale counts on random samples of leaves and fruits. At intervals during each season counts were made of the number of leaves on 100 terminal shoots, 10 to 12 in. long and bearing 13 to 16 leaves each, taken at random in each orchard, and counts were also taken of scab lesions on these leaves. The results accord well with those obtained by the other methods of sampling (Table 1). On June 19, 1937, the indicated reduction in the incidence of scab lesions on the leaves of terminal shoots in the sprayed orchard was 91 per cent.

TABLE 1.—*Effects of a fall application of an eradicant fungicide on the incidence of perithecia of Venturia inaequalis and the development of scab in the following season, Wealthy variety, Madison, Wis., 1936–1939*

Date	Counts made of[a]	Av. lesions per spur, terminal leaf, or fruit in nonsprayed orchard	Indicated reduction in incidence of perithecia or lesions by the spray
		Number	*Per cent*
1937			
May 20	Perithecia in overwintered leaves (100–100)[b]	......	97
May 25	Lesions on leaves of spurs (50–50)	0.0	....
May 27	do	0.1	100
May 29	do	0.3	100
May 31	do	0.5	100
June 4	do	0.6	100
June 6	do	0.7	97
June 18	Lesions on leaves of terminals (100–100)	0.3	91
Sept. 15	do	10.7	96
June 19	Lesions on fruits (200–200)	0.2	100
July 13	do	0.4	84
July 28	do	0.3	85
Aug. 26	Lesions on fruits (1000–1000)	0.3	96
1938			
May 6	Perithecia in overwintered leaves (100–100)[b]	......	100
May 14	Lesions on leaves of spurs (50–50)	0.0	....
May 17	do	0.2	100
May 20	do	0.3	100
May 23	do	0.4	96
May 26	do	0.6	97
May 29	do	0.9	96
June 1	do	1.0	96
June 4	Lesions on leaves of terminals (100–100)	3.2	96
June 16	do	11.3	85
June 28	do	16.5	80[c]
June 4	Lesions on fruits (300–300)	1.8	97
June 16	do	3.3	91
June 28	do	4.9	87
July 11	do	6.7	88
July 23	do	7.5	91
Aug. 4	do	12.4	92
Aug. 17	do	12.5	92
Aug. 30	do (600–500)	15.5	91
1939			
May 19	Perithecia in overwintered leaves (100–100)[b]	....	98
May 19	Lesions on leaves of spurs (50–50)	0.0	....
May 22	do	0.3	100
May 25	do	0.6	100
May 28	do	2.1	99
May 31	do	3.0	99
June 3	do	4.1	99
June 6	do	4.3	99
May 29	Lesions on leaves of terminals (100–100)	0.5	99
June 12	do	3.7	97
June 26	do	12.9	96
May 29	Lesions on fruits (1200–300)	0.2	100
June 12	do	2.4	99
June 26	do	4.2	98
July 10	do	4.6	98
Aug. 1	do	5.2	98
Sept. 1	do (2000–1000)	3.3	98

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[a] The first number in parentheses refers in each case to the units examined from the sprayed orchard, the second to those from the nonsprayed. For spray treatment see text.

[b] The average numbers of perithecia per sq. in. of surface of nontreated leaves examined were: 1936–37, 2.5; 1937–38, 15.8; 1938–39, 25.5. Counts of the perithecia were taken on 100 leaves collected at random from each, the nontreated and the treated, orchard.

[c] Ascospores, blown in from a nearby nursery and the nontreated orchard, are thought to have accounted for a large part of the scab in the nonsprayed orchard in 1938 (see text).

Through most of June it ranged from 96 to 80 per cent in 1938 and 99 to 96 per cent in 1939. It is noteworthy that scab development was very sparse in 1937, severe in 1938, and moderate in 1939. Except in 1937, comparative counts of leaf lesions could not be made satisfactorily after late June, because the lesions on the more severely diseased leaves became confluent.

Counts of the number of scab lesions on 200 to 2,000 fruits taken at random in each, the sprayed and the nonsprayed orchard, were made at intervals from early June to late August. The data, which are shown in table 1, indicate that the eradicant spray treatment reduced the incidence of scab lesions on the sprayed fruits on the several dates by 100 to 84 per cent in 1937, 97 to 87 per cent in 1938, and 100 to 98 per cent in 1939, in comparison with that on the nonsprayed fruits. The dropping of badly scabbed fruits accounts in large measure for the minor irregular fluctuations of the data.

TABLE 2.—*Effect of a fall application of an eradicant fungicide on the control of apple scab on Wealthy fruit, Madison, Wis., 1936–1939*[a]

Year, orchard, and treatment	Fruits examined	Scabbed fruits[b]				Average lesions per fruit	Indicated reduction in incidence of lesions by the spray
		Slight	Commercial	Bad	Total		
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Number*	*Per cent*
1937							
Orchard 2, nonsprayed	1000	4	3	2	8.0	0.3	......
Orchard 1, sprayed	1000	0	0	0	0.5	0.0	96
1938							
Orchard 2, nonsprayed	600	9	12	71	92.0	15.5	......
Orchard 1, sprayed	500	13	7	9	29.0	1.3	91[c]
1939							
Orchard 2, nonsprayed	1000	31	17	18	66.0	3.3	......
Orchard 1, sprayed	2000	3	1	0	4.0	0.1	98

[a] Data taken at harvest: Aug. 26, 1937; Aug. 30, 1938; Sept. 1, 1939. For spray treatment see text.

[b] The scabbed fruits were classed as "slight" (1 to several small spots); "commercial" (worse than "slight," but marketable); and "bad" (not marketable). A more detailed account of these classes has been published (6).

[c] See footnote *c*, table 1.

At harvest, records were made of the occurrence of scab on 500 to 2,000 fruits taken at random in each, the nonsprayed and the sprayed, orchard. The data (Table 2), indicate that the spray reduced the incidence of scab lesions on the Wealthy fruits at harvest by 96, 91, and 98 per cent, respectively, in 1937, 1938, and 1939.

In a previous paper (7) it was shown that ascospores blown from the nontreated to the treated orchard contributed substantially to the development of scab in the treated, this effect diminishing rapidly with increased distance. Accordingly, data in the sprayed orchard were taken separately in the 4 100-ft. distance classes, successively more remote from the nonsprayed orchard. In 1938, it was noted in the earlier counts that variation in the incidence of scab in the distance classes showed the reverse of the expected trend. Examination of a young apple nursery (Fig. 1) that had recently been established nearby showed that the overwintered leaves bore abundant perithecia of the scab fungus. The effect of inoculum blown into the sprayed orchard from this source is shown by the data in table 3.

TABLE 3.—*Incidence of scab lesions in the treated orchard in relation to distance from an extraneous source of ascosporic inoculum in the nearby apple nursery (Fig. 1). (Data taken on Wealthy fruit on August 30, 1938.)*

Distance class	Fruits examined	Average lesions per fruit	Indicated reduction in incidence of lesions
Feet from nursery	*Number*	*Number*	*Per cent*
100–200	102	5.1	0[a]
200–300	372	2.9	43
300–400	500	2.2	57
400–500	500	1.3	75

[a] The average number of lesions per fruit in this distance class is used as the basis for computing percentage reduction.

The development of scab in the sprayed orchard in 1938 was, therefore, influenced by ascospores blown in at different times from this nursery and from the nonsprayed orchard. Since the dominant influence came from the nursery, the data shown in figure 3 and tables 1 and 2 from the sprayed orchard in 1938 were taken from the distance class farthest from this extraneous source of inoculum. It is noteworthy that, when an eradicant ground spray was applied in the nursery in the spring of 1939, the influence from the nursery on the development of scab in the treated orchard was eliminated, and the indicated reduction of scab in this orchard by the treatment corresponded very closely to the indicated reduction in incidence of perithecia.

Records were taken each summer on the percentage of dead lateral and terminal buds on the sprayed and the nonsprayed trees. No significant injury attributable to the spray was observed on Wealthy. Some injury to both lateral and terminal buds was observed on Northwestern Greening and McIntosh. No conclusions are drawn with reference to whether the fall spray treatment employed in this experiment is safe enough for use in commercial orchards. The facts that some bud injury occurred on Northwestern and McIntosh trees and that injury from such treatments may vary with the variety used and the conditions encountered lead us to withhold recommendations relating to this type of fall spraying, pending further studies. The encouraging results from ground spray treatments reported in a later section of this paper and the fact that the spray injury problem may be largely avoided by treating the dead leaves on the ground have led to increased emphasis on that aspect of the work.

Discussion

The 3 years' data presented herein agree with those from the same orchards in the two preceding years (7) in showing that drastic suppression of perithecia of *Venturia inaequalis* by a fall application of eradicant fungicide resulted in drastic reduction in the occurrence of scab in the ensuing season. It has been pointed out above that the data on incidence of scab lesions on the leaves of fruit spurs (Table 1) furnish an excellent index of ascosporic infection, and that the indicated reduction in incidence of lesions on these leaves in the treated orchard, as compared with the nontreated, is in approximately direct relation to the corresponding reduction in perithecia. This correspondence, however, was not confined to ascosporic infection. It held to a remarkable degree, though not quite so closely, throughout the season for general leaf and fruit infection. The data taken at harvest (Table 2), for example, show the following percentage reductions in number of lesions per fruit for the treated orchard, as compared with the nontreated: 1936–37, 96; 1937–38, 91; and 1938–39, 98. It should be remembered that in 1938 ascospores, blown in from the nontreated nursery, played an important part in the development of scab in the treated orchard.

Attention is invited to the fact that it was not the objective of these experiments to attain control of scab by an eradicant treatment alone. Summer spraying was omitted in order to facilitate evaluation of the effects of eradicant spraying on the epidemiology and control of the disease. The high degree of control that resulted from the eradicant treatments gives much encouragement for their further development. However, it should not be assumed that this degree of control can be obtained by similar eradicant treatments on every variety or under all seasonal conditions. The Wealthy variety, which was available for these experiments, is only moderately susceptible to scab, and its fruit attains a fairly high degree of resistance in the latter part of the season. With the more susceptible later varieties, such as McIntosh, experience has shown that, without summer spraying, scab may build up from a very small early-season infection to a severe epidemic, if weather conditions are very favorable. Furthermore, while some very favorable conditions for scab development have been encountered in the work that has been reported, still more favorable conditions may occur. The evidence that has been presented, however, shows conclusively that reductions in the ascosporic inoculum to the levels reached in these experiments greatly retard and reduce development of the epidemic.

ERADICANT FUNGICIDAL TREATMENT OF LEAVES ON THE GROUND

In situations in which the overwintering of the scab fungus depends wholly or dominantly on production of the perithecial stage, as is the case in most apple-growing areas of the United States, the period after leaf-fall and before ascospore discharge begins in the following spring is a potentially vulnerable one (2, 7). Much more drastic chemical treatments can then be applied to the fallen leaves than would be tolerated by the tree in treatments after harvest and before leaf-fall. For several years increased emphasis has been placed on this phase of the work. The earlier results have been reported and pertinent literature has been reviewed (7).

Comparison of Fungicidal Materials

Materials and Methods. As comparatively little information was available concerning the effectiveness of materials for our purpose some small-scale exploratory experiments were performed. The following materials were used.

Aerosol.—A proprietary emulsifier.

Ammonium sulphate.—A finely ground or pellet form of a commercial product used as a fertilizer.

Copper sulphate.—Same as listed on page [3].

Elgetol Extra.—Same as listed on page [3].

Ethyl mercury oleate.—A proprietary fungicide containing 10 per cent of ethyl mercuric oleate.

Formalin.—A commercial product containing 40 per cent formaldehyde in aqueous solution.

Hydrated lime.—Same as listed on page [3].

Kerosene emulsion.—Commercial kerosene emulsified by means of Aerosol.

Lignasan.—A proprietary fungicide containing 6.25 per cent ethyl mercuric phosphate.

Monocalcium arsenite.—Same as listed on page [3].

IN–181–P.—A proprietary emulsifier.

Sodium arsenite.—A commercial solution containing sodium arsenite equivalent to 4 lb. white arsenic (As_2O_3) per gallon.

Sulphuric acid.—A commercial grade, sp. gr. 1.84.

Two methods of treating overwintered leaves, bearing abundant perithecia, were employed. In 1937 and 1938 samples of such leaves (McIntosh or Dudley) were collected at random and placed in open-mesh cloth bags (7). These were then sprayed with various materials. Duplicate bags were used for each treatment. In 1937, 1938, and 1939 overwintered leaves on 400-sq.-ft. plots in a sod orchard were treated with various materials by means of a wheelbarrow sprayer that maintained a pressure of about 150 lb.

When most of the ascospores had matured, the bags of leaves and samples of leaves taken at random from the treated plots in the orchard were air-dried and stored pending examination. In 1937 the leaves were stored in the laboratory and examined in October, when it was found that satisfactory evaluation of the material could not be made by microscopic examination of crushed perithecia, as had been planned. In 1938 and 1939 the air-dry samples were stored at about 6° C., and laboratory studies of ascospore discharge were made in June and early July. From each sample 20 of the leaves bearing perithecia most abundantly were selected and a representative disk .5 inch in diameter was cut from each. These disks were then wet and placed adherent to the glass within the tops of Petri dishes, with the dorsal surface oriented towards a water-agar gel contained in the bottoms. The dishes were held for 6 hours at 12° C.; counts were then made of the ascospores caught on the agar surfaces. If the discharge was light all the spores were counted; if moderate or heavy, the counts were made in 8 areas taken at random under each disk, each area being 1/100 that of the disk. The average number of spores discharged per disk was computed.

Experimental Results. The treatments and results of the small-scale experiments in 1938 and 1939 are shown in tables 4 and 5.

The bagged leaves were treated in 3 replicated series, each series being sprayed on a different date so that there might be variation in relation of time of treatment to rain periods (Table 4, footnote *d*). Treatments No. 2, 5, 9, and 10 were highly effective, regardless of the timing, whereas No. 8 was relatively ineffective in all series. Treatments 1, 3, 4, 6, and 7 gave variable results, all being distinctly lessened in effectiveness under the conditions encountered by Series 2, in which fairly heavy rains fell 8 and 30 hours, respectively, after the spray was applied.

Three of the 4 treatments (H_2SO_4, 10 per cent, and Formalin, 1 and 2 per cent, respectively) that gave consistently good results in all three series with bagged leaves gave poor results in the orchard-plot experiments (Table 5, plots 2, 9, and 10). The fourth material (the acidified preparation of copper sulphate and calcium and sodium arsenites), however, showed a high degree of efficiency when applied to leaves on the orchard floor (plots 5 and 23), as well as to those in bags. The similar mixture used on plot 24 was also highly efficient. The treatments of plots 1, 3, 4, 6, 7, and 8 were comparatively inefficient.

While mixtures of copper sulphate with arsenites showed a high degree of effectiveness, certain organic materials were distinctly more effective, and attention has been directed increasingly to them. Elgetol Extra at the 1 per cent concentration gave an indicated reduction of ascospore discharge of 99.3 per cent (plot 13). At the increased dilutions (plots 14 and 15), the indicated effectiveness was substantially reduced. In view of other experience, however, and because of the fact that 80 per cent of the spores discharged from the 20 disks from plot 14 came from a single disk, it is thought that the indicated reduction by the .5 per cent dilution is unduly low. Elgetol Extra, .3 per cent, plus monocalcium arsenite, .5 per cent, was highly efficient (plot 16), but other combinations of dilute Elgetol Extra with supplements were less efficient (plots 17, 18, and 19).

The results from Lignasan were excellent in one plot (No. 21) and inferior in its duplicate (No. 20). It is thought that the superior result is more reliable, since 90 per cent of the spores came from 2 of the 20 disks from plot 20. Under the conditions of this plot work in 1939 there was some possibility that leaves might be blown from a nontreated to a treated area. A question, therefore, must attach to results in which one or two

TABLE 4.—*Effectiveness of eradicant fungicidal treatments of overwintered apple leaves for reduction of the ascosporic inoculum of Venturia inaequalis, Sturgeon Bay, Wis., 1938. (Samples of leaves were placed in cloth-mesh bags on sod and treated.)*

Treatment No.	Spray treatment[a]	Average number of ascospores discharged per disk[b]			Indicated reduction in ascosporic inoculum by treatment[c]		
		Series 1[d]	Series 2	Series 3	Series 1	Series 2	Series 3
		Number	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
1	$(NH_4)_2SO_4$ 12.5	410	1550	11	93	73	100
2	H_2SO_4 10	10	45	0	100	99	100
3	CS 1 + L .1 + Nai .66 + H_2SO_4 .08	301	1221	646	95	78	88
4	CS 1 + Nai .66 + H_2SO_4 .08	425	640	25	92	89	100
5	CS 1 + Cai .5 + Nai .4 + H_2SO_4 .08	8	7	0	100	100	100
6	$(NH_4)_2SO_4$ 12.5 + Nai .2 + H_2SO_4 .08	210	1207	375	96	79	93
7	Nai .66	162	1580	24	97	72	100
8	CS 1	1670	2124	1760	70	59	69
9	Formalin 2	0	20	0	100	100	100
10	Formalin 1	0	135	0	100	98	100
11	Nontreated	5630	5630	5630	0	0	0
12	H_2SO_4 5	......	......	351	......	......	94

[a] CS = copper sulphate; L = hydrated lime; Cai = monocalcium arsenite; Nai = sodium arsenite. The numbers refer to the percentage by weight, assuming the mixture to have the same weight as water, with the exception of formalin, for which they refer to gallons per 100 gallons.

[b] See text for method.

[c] The number of ascospores discharged from the nontreated leaves was used as the basis for computing percentage reduction.

[d] Series 1, sprayed April 26, two days prior to a rain; leaves were thoroughly dry. Series 2, sprayed on May 2, following .05 inch rain when leaves were still wet, 8 hours prior to .65 inch rain, and 30 hours prior to another .91 inch rain. Series 3, sprayed on May 5, following .06 inch rain when leaves were still wet; leaves dried rapidly and on May 9 a 16-hour rain of .70 inch occurred. All the leaves were taken up May 12 for ascospore-discharge studies.

TABLE 5.—*Effectiveness of eradicant fungicidal treatments of overwintered apple leaves for reduction of the ascosporic inoculum of Venturia inaequalis, Sturgeon Bay, Wis., 1938 and 1939. (Plots 20 × 20 feet in a sod apple orchard were sprayed at the rate of 600 gal. per acre on April 21, 1938, and April 19, 1939.)*

Plot No.	Dates of collection of leaf samples,[a] variety, and treatment[b]	Average ascospores discharged per disk[c]	Indicated reduction in ascosporic inoculum by treatment[d]
		Number	*Per cent*
	May 12, 1938 (McIntosh)		
1	$(NH_4)_2SO_4$ 12.5	516	84.2
2	H_2SO_4 10	713	78.2
3	CS 1 + L .1 + Nai .66 + H_2SO_4 .08	1126	65.5
4	$(NH_4)_2SO_4$ 12.5 + Nai .2 + H_2SO_4 .08	995	69.5
5	CS 1 + Cai .5 + Nai .4 + H_2SO_4 .08	97	97.0
6	CS 1 + Nai .66 + H_2SO_4 .08	432	86.8
7	Nai .66	596	81.8
8	CS 1	2437	25.3
9	Formalin 2	1775	45.6
10	Formalin 1	1287	60.6
11	Untreated	2560	0
11a	"	3969	
	May 23, 1939 (Dudley)		
12	Untreated	6269	....
13	Elgetol Extra 1	45	99.3
14	" " .5	582	90.7
15	" " .3	1767	71.8
16	" " .3 + Cai .5	111	98.2
17	" " .3 + Cai .25	398	93.7
18	" " .1 + Cai .5	3835	38.8
19	" " .3 + CS 1 + L .25 + Cai .75	485	92.3
20	Lignasan .125 + IN–181–P .03	1000	84.0
21	do	164	97.4
22	Ethyl mercury oleate .125	2012	67.9
23	CS 1 + Cai .5 + Nai .5 + H_2SO_4 .08	297	95.3
24	CS .25 + Nai 1 + Kerosene Emulsion 4	238	96.2
	June 21, 1939 (Dudley)		
12	Nontreated	104	....
13	Elgetol Extra 1	0	100.0
16	" " .3 + Cai .5	4	95.9
21	Lignasan .125 + IN–181–P .03	1	99.5

[a] Samples were collected at random from uncovered leaves.

[b] see footnote *a*, table 4. The dosage of Elgetol Extra is expressed in per cent by volume.

[c] See text for method.

[d] See footnote *c*, table 4.

leaves of the sample furnished a high percentage of the spores. This difficulty is being obviated in further similar experiments by covering the sampling areas with wire netting.

Ethyl mercury oleate was comparatively inefficient in this test (plot 22).

To gain evidence on whether the seemingly effective treatments might merely have delayed the discharge of ascospores, samples were taken on June 21 from plots 12, 13, 16, and 21. Tests of these (Table 5) showed little or no discharge from the treated samples and very sparse discharge from the nontreated. Microscopic examination of both fresh material and histological preparations confirmed these results.

It is recognized that data on ascospore discharge from a larger number of leaves per sample would have been desirable, and an effort is being made to develop a more adequate method for evaluating the quantity of the ascosporic inoculum. However, the results thus far obtained have been highly consistent, and are thought to be reliable for the cases of nearly complete suppression of ascospore discharge. They do not accurately reveal differences in effectiveness of inferior treatments, but little interest attaches to such differences.

Effects of a Ground Spray of Elgetol Extra on the Epidemiology and Control of Scab in a Commercial Orchard

Elgetol has been recommended for dormant spraying of trees and vines to combat various fungus diseases (10, 11). Small-scale experiments with Elgetol Extra were performed in the spring of 1938 to test its effectiveness in suppressing the ascosporic inoculum of *Venturia inaequalis* when sprayed on overwintered apple leaves on the ground. The results (3) were so promising that this material at a dilution of 1 part by volume in 100 was used for treating the leaves on the ground in a commercial orchard in the spring of 1939. Certain orchards that received no ground spray were studied comparatively.

Materials and Methods.

Experimental orchards: Stephenson's.—A 10-acre sod orchard of McIntosh, Delicious, and Dudley, about 20–25 years old. Not more than a total of 8 acres of other plantings of apples within a .75-mile radius, including 5-acre orchard (Samuelson's) .3 mile, 1-acre orchard .2 mile, and 1-acre orchard .1 mile away.

Samuelson's.—A 5-acre orchard, chiefly McIntosh and Delicious, about 20–25 years old, .3 mile north of Stephenson orchard. Cultivated, except for blocks about 6 ft. square at bases of the trees.

Goldman's.—A 6-acre block of McIntosh trees about 25 years old in 115-acre sod apple orchard located about 4 miles north of Stephenson orchard.

Sackett's.—A 5-acre Wealthy and Dudley sod orchard 29 years old located .5 mile north of Goldman orchard.

All the experimental orchards were located on gently rolling to rolling ground. Soil sandy loam or silt loam. There was a substantial development of scab on the foliage in all the experimental orchards in 1938.

Ground spray treatments.—On April 22–23, 1939, leaves on ground in a 6-acre block of McIntosh trees in Stephenson orchard, sprayed with Elgetol Extra, 1 per cent, at 450 gal. per acre. Remainder of orchard, treated with Elgetol Extra, .5 per cent, plus monocalcium arsenite, .5 per cent. Two operators using double-nozzle guns applied spray to area between 2 rows as they followed power spray rig, which maintained approximately 400 lb. pressure. Discharges from the guns overlapped. The ground in a zone 50 to 100 ft. wide around edge of orchard, sprayed to treat leaves blown out of orchard. No ground treatment applied in Sackett, Goldman, and Samuelson orchards.

Summer spray treatments.—Spray applied with guns from top of power rigs. Certain applications omitted on some trees in Stephenson and Sackett orchards, to obtain evidence on severity of primary infection and effectiveness of reduced spray programs in treated and nontreated orchards, respectively. A few trees in each orchard left unsprayed for study of the development of the disease during the season (Table 10).

Epidemiological Records. Continuous records of rainfall, temperature, relative humidity, wind direction, wind velocity, and hours of bright sunshine were taken by standard meteorological instruments set up in the Sackett orchard (6). The daily total precipitation, maximum and minimum

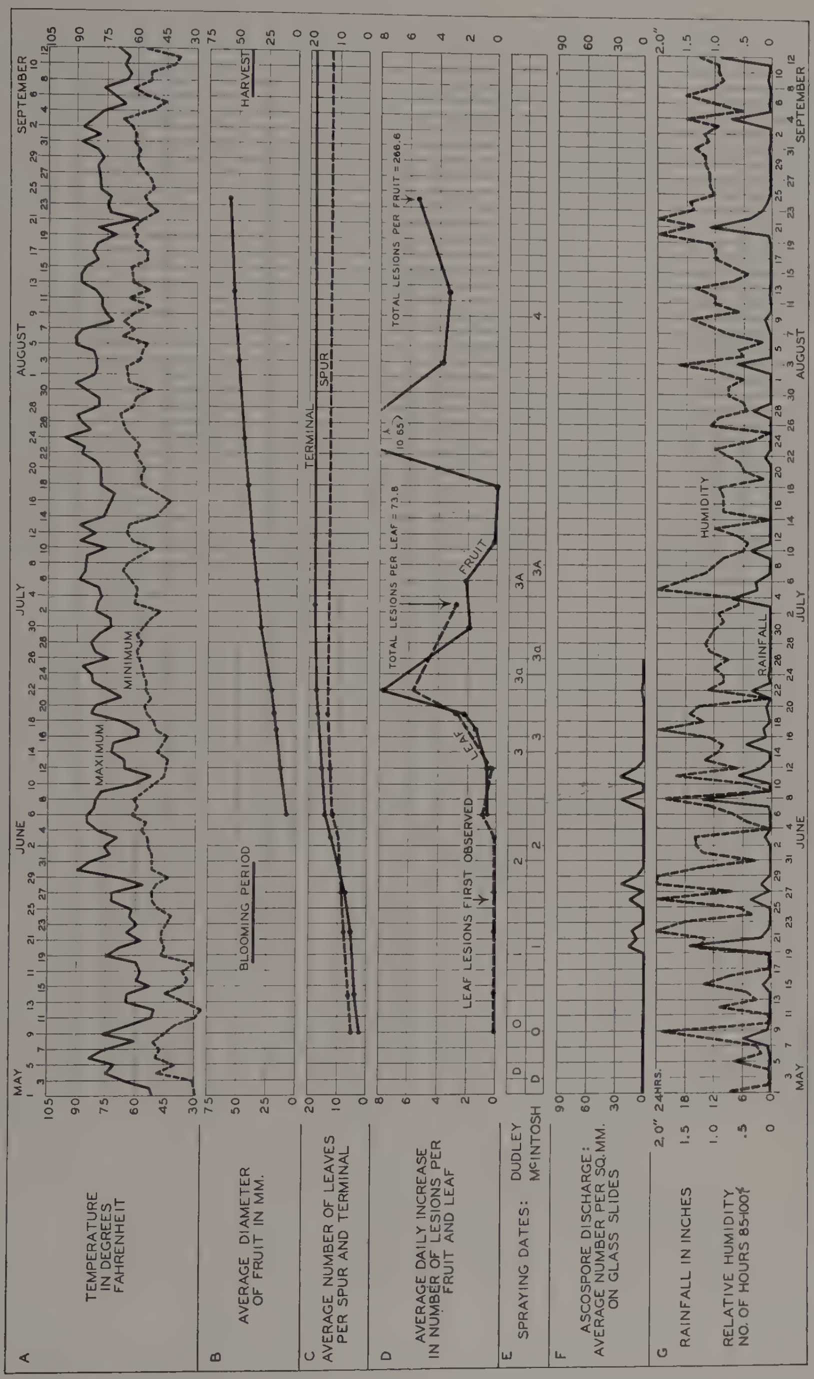

FIG. 5. Summary of certain records relating to the epidemiology and control of apple scab, Sturgeon Bay, Wis., 1939. (See text.)

temperatures, and number of hours of relative humidity of 85–100 per cent are shown in figure 5, along with epidemiological data taken on the Dudley variety in the Sackett orchard by the general methods that have been described earlier (6). (The data on scab development were taken on leaves of 20 tagged spurs, 20 tagged terminals, and 50 tagged fruits. The dates of spray applications are for Dudley at Sackett's and McIntosh at Goldman's.) The season was one of well-distributed rain periods, moderate temperature, and unusually severe scab development.

Experimental Results. To study the effect of the eradicant treatment in reducing the ascosporic inoculum in the Stephenson orchard, overwintered leaves were collected and the ascospores discharged from them. For sampling purposes the orchard was divided into 3 approximately equal areas. Prior to the treatment, about 500 exposed leaves (not covered by other leaves) were collected at random over each area and placed in open-mesh bags, laid on sod in the Sackett orchard until May 24. On May 24 similar samples of exposed leaves were collected at random from each of the sampling areas. The samples collected before and after treatment, respectively, were stored air-dry at about 6° C. and tested for ascospore discharge during June and early July by the method described on page 14. The results (Table 6) indicate that the treatment with Elgetol Extra at the 1 per cent concentration reduced the ascosporic inoculum from the exposed leaves by about 96 per cent. The close agreement of results from the 3 sampling areas is noteworthy.

TABLE 6.—*Tests of the effectiveness of an eradicant treatment in limiting the ascosporic inoculum of V. inaequalis in a commercial McIntosh orchard, Sturgeon Bay, Wis., 1939. (Elgetol Extra, 1-per cent, was applied Apr. 22–23 to overwintered leaves on the ground in the Stephenson orchard at the rate of 450 gal. per acre.)*

Area[a] number	Average ascospores discharged per disk of leaf surface[b]		Indicated reduction in incidence of discharged ascospores by treatment
	Samples collected before treatment	Samples collected after treatment	
	Number	*Number*	*Per cent*
1	2181	61	97.2
2	2796	151	94.6 Av. 96.4
3	2215	55	97.5

[a] For sampling purposes the orchard was divided into 3 approximately equal areas.
[b] See text for method.

Samples were taken before and after treatment from leaves in drifted heaps at depths to which the fungicide did not penetrate. Uninjured perithecia were found in fair abundance in all these collections. While this aspect of the problem requires further study, the high degree of effectiveness of the treatment in delaying and reducing the intensity of scab development, as shown below, indicates that, under the conditions of this experiment, ascospores from these underlying leaves that escaped the fungicide could not have played any important rôle in initiating the disease. It seems probable that the overlying leaves prevented effective dissemination of these spores.

Comparative data on sepal infection were taken on June 6–8 on nonsprayed trees in the Stephenson, Samuelson, and Sackett orchards. Examination was made of 100 to 200 fruits taken at random from each of 1 to 6 trees per variety in each orchard (Table 7).

TABLE 7.—*Relation of eradicant fungicidal treatments to incidence of sepal infection, Sturgeon Bay, Wis., June 6–8, 1939*

Variety, orchard, and treatment[a]	Fruits examined	Fruits showing sepal infection	Indicated reduction in incidence of sepal infection on fruits by treatment[b]
	Number	*Per cent*	*Per cent*
McIntosh (Stephenson), Eradicant treatment E.E. 1; no summer spray	1200	0.3	99
Delicious (Stephenson), Eradicant treatment E.E. .5 + Cai .5; no summer spray	200	2.0	95
McIntosh (Samuelson), No eradicant treatment No summer spray	200	37.0	......
Delicious (Samuelson), No eradicant treatment No summer spray	200	40.0	......
Dudley (Sackett), No eradicant treatment No summer spray	200	40.0	......
Wealthy (Sackett), No eradicant treatment No summer spray	200	33.5	......

[a] E.E. = Elgetol Extra (dosage expressed in per cent by volume); Cai = monocalcium arsenite (dosage expressed in per cent by weight). The eradicant sprays were applied April 22–23.

[b] Comparison is made of the data from nonsprayed trees in the Stephenson (treated) and Samuelson (nontreated) orchards.

The incidence of sepal infection on nonsprayed trees at this time is a very good index of the comparative effectiveness of the ascosporic inoculum in the different orchards, as no secondary infection had appeared. In the treated orchard (Stephenson's) .3 per cent of the nonsprayed McIntosh fruits and 2 per cent of the nonsprayed Delicious showed sepal infection, as compared with 37 and 40 per cent, respectively, in the nontreated orchard (Samuelson's). The indicated reductions in this sepal infection by the treatment, assuming other conditions to be alike in the two orchards, are 99 and 95 per cent, respectively, for the two varieties. These figures agree closely with the indicated reduction in effective ascosporic inoculum (Table 6). It is noteworthy that sepal infection on Dudley and Wealthy in the Sackett orchard, which received no eradicant fungicidal treatment, was very similar in amount to that on McIntosh and Delicious in the Samuelson orchard.

The development of scab on the foliage was followed until about 3 weeks after petal-fall on nonsprayed McIntosh trees in both the treated (Stephenson) and the nontreated (Samuelson) orchards. At intervals of 4 to 12 days data were taken on tagged fruit spurs and terminals, 5 of each on 1 tree in the nontreated orchard and 20 of each on 2 trees in the treated. Similar

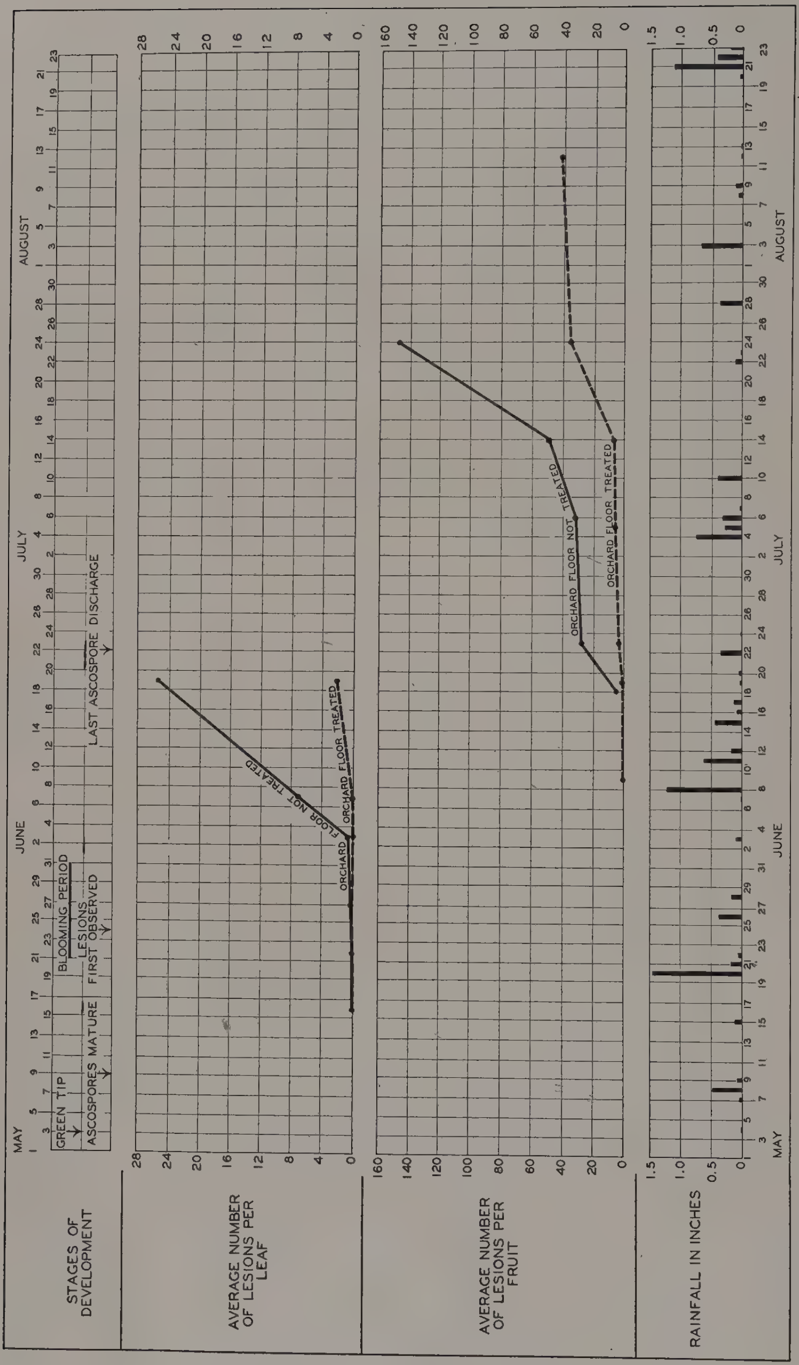

FIG. 6. Summary of certain records relating to the epidemiology and control of apple scab in the Stephenson (Elgetol-treated) and Samuelson (nontreated) orchards, Sturgeon Bay, Wis., 1939. (See text.)

records of scab development were taken on 25 tagged fruits on each of the trees on which the records of leaf phase of the disease were taken.

The data (Fig. 6) indicate that on June 19 there was 91 per cent less scab per leaf in the treated than in the nontreated orchard; similarly, on July 14 there was 88 per cent less scab per fruit.

Further data on the effects of the eradicant treatments on the development and control of scab were recorded from leaves and fruits taken at random in parts of the trees that could be reached from the ground. Usually 10 terminals and 25 fruits were examined per tree. In cases in which the number of trees was small a larger number of terminals or fruits per tree was examined.

The results (Table 8) show that on June 9 there were 98.8 per cent fewer lesions on leaves of nonsprayed McIntosh trees and 99.9 per cent fewer on

TABLE 8.—*Effect of ground spraying with eradicant fungicides on the development of apple scab on the foliage, Sturgeon Bay, Wis., 1939*

Date, variety, orchard, and treatment[a]	Total leaves examined[b]	Leaves infected	Average lesions per leaf	Indicated reduction in incidence of lesions by treatment[c]
	Number	*Per cent*	*Number*	*Per cent*
June 9				
McIntosh (Stephenson), Eradicant treatment				
E.E. 1; no summer spray	1258	4.3	0.08	98.8
E.E. 1; L–S., 1–40, D, 0, 1, 2	1167	0.1	0.00	....
E.E. 1; L–S., 1–40, 0, 1, 2	1269	0.7	0.01	....
E.E. 1; L–S., 1–40, 1, 2	1238	0.4	0.00	....
E.E. 1; L–S., 1–40, 2	1287	1.2	0.02	....
Delicious (Stephenson), Eradicant treatment				
E.E. .5 + Cai .5; no summer spray	658	0.6	0.01	99.9
McIntosh (Samuelson), No eradicant treatment				
No summer spray	275	72.0	6.95	....
Delicious (Samuelson), No eradicant treatment				
No summer spray	295	71.5	4.84	....
July 11				
McIntosh (Stephenson), Eradicant treatment				
E.E. 1; no summer spray	770	79.2	25.80	68.8
E.E. 1; L–S., 1–40, D, 0, 1, 2; 1–60, 3; 1–75, 3A	1439	8.2	0.16	....
E.E. 1; L–S., 1–40, 0, 1, 2; 1–60, 3; 1–75, 3A	1437	15.6	0.52	....
E.E. 1; L–S., 1–40, 1, 2; 1–60, 3; 1–75, 3A	1496	20.3	0.95	....
E.E. 1; L–S., 1–40, 2; 1–60, 3; 1–75, 3A	806	27.3	1.39	....
McIntosh (Samuelson), No eradicant treatment				
No summer spray	331	99.0	82.48	....

[a] See footnote *a*, table 10.

[b] All leaves on 25, 50, or 100 terminals taken at random on 1 to 10 trees per plot were examined.

[c] See footnote *b*, table 7.

TABLE 9.—*Effects of ground spraying with eradicant fungicides on the development of apple scab on fruits, Sturgeon Bay, Wis., 1939*

Variety, orchard, and treatment[a]	Fruits examined	Scabbed fruits on stated dates			Average lesions per fruit on stated dates		
		June 20–21	July 24–25	Aug. 17–23	June 20–21	July 24–25	Aug. 17–23
	Number	*Per cent*	*Per cent*	*Per cent*	*Number*	*Number*	*Number*
McIntosh (Stephenson), Eradicant treatment							
E.E. 1; no summer spray	200–300	17	92	95	0.56	21.75	38.80
E.E. 1; L–S., 1–40, D, 0, 1, 2; 1–60, 3; 1–75, 3A; 1–50, 4	600–1500	0	0	1	0.00	0.01	0.03
E.E. 1; L–S., 1–40, D, 0, 1, 2; 1–60, 3; 1–75, 3A	300	......	0	5	......	0.01	0.07
E.E. 1; L–S., 1–40, 0, 1, 2; 1–60, 3; 1–75, 3A; 1–50, 4	300	0	2	1	0.00	0.02	0.01
E.E. 1; L–S., 1–40, 1, 2; 1–60, 3; 1–75, 3A; 1–50, 4	300	1	2	2	0.01	0.02	0.03
E.E. 1; L–S., 1–40, 2; 1–60, 3; 1–75, 3A; 1–50, 4	300–1000	2	9	9	0.08	0.35	0.30
Delicious (Stephenson), Eradicant treatment							
E.E. .5 + Cai .5; no summer spray	100	21	29	90	0.41	0.95	5.34
McIntosh (Samuelson), No eradicant treatment							
No summer spray	50	100	100	......	26.00	......	......
Delicious (Samuelson), No eradicant treatment							
No summer spray	50	100	......	......	20.04	......	......
Dudley (Sackett), No eradicant treatment							
No summer spray	50	100	100	100	35.30	130.92	165.00
L–S., 1–50, D, 0, 1; 1–60, 2, 3, 3a, 3A	100–200	2	4	4	0.01	0.05	0.09
L–S., 1–50, D, 0, 1; 1–60, 2, 3, 3A	100	......	9	......	......	0.25	......
L–S., 1–50, 0, 1; 1–60, 2, 3, 3a, 3A	100–200	12	4	5	0.12	0.12	0.07
L–S., 1–50, 1; 1–60, 2, 3, 3a, 3A	100–200	17	9	10	0.18	0.12	0.27
L–S., 1–60, 2, 3, 3a, 3A	100–200	81[b]	61	50	4.05	2.71	1.99
Wealthy (Sackett), No eradicant treatment							
No summer spray	100	100	......	......	15.25	......	......

[a] See footnote *a*, table 10.

[b] Many of the badly scabbed fruits dropped early, thus accounting for the seeming decrease in the amount of scab in later counts.

TABLE 10.—*Effect of ground spraying with eradicant fungicides on the control of apple scab at harvest, Sturgeon Bay, Wis., 1939*

Variety, orchard, and treatment[a]	Count trees	Fruits examined	Scabbed fruits[b] Slight	Com-mercial	Bad	Total
	Number	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
McIntosh (Stephenson), Eradicant treatment						
E. E. 1; no summer spray	5	1103	2	6	92	100
E.E. 1; L–S., 1–40, D, 0, 1, 2; 1–60, 3; 1–75, 3A; 1–50, 4	22	2218	1	0	0	1
E.E. 1; L–S., 1–40, 0, 1, 2; 1–60, 3; 1–75, 3A; 1–50, 4	10	1090	4	1	1	6
E.E. 1; L–S., 1–40, 1, 2; 1–60, 3; 1–75, 3A; 1–50, 4	9	1057	4	1	1	5
E.E. 1; L–S., 1–40, 2; 1–60, 3; 1–75, 3A; 1–50, 4	5	1635	5	5	5	15
E.E. 1; L–S., 1–40, D, 0, 1, 2; 1–60, 3; 1–75, 3A	6	900	23	4	1	27
E.E. .5 + Cai .5; L–S., 1–40, D, 0, 1; 1–60, 3; 1–75, 3A; 1–50, 4	10	712	8	3	4	15
Delicious (Stephenson), Eradicant treatment						
E.E. .5 + Cai .5; no summer spray	2	227	29	36	34	99
E.E. .5 + Cai .5; L–S., 1–40, D, 0, 1, 2; 1–60, 3; 1–75, 3A; 1–50, 4	10	1048	1	1	0	2
E.E. .5 + Cai .5; L–S., 1–40, D, 0, 1; 1–60, 3; 1–75, 3A; 1–50, 4	10	1055	2	2	1	5
Dudley (Stephenson), Eradicant treatment						
E.E. .5 + Cai .5; L–S., 1–40, D, 0, 1, 2; 1–60, 3; 1–75, 3A	10	1000	0	0	0	0
McIntosh (Samuelson), No eradicant treatment						
No summer spray	1	65	0	0	100	100
Delicious (Samuelson), No eradicant treatment						
No summer spray	1	215	0	0	100	100
McIntosh (Goldman), No eradicant treatment						
No summer spray	4	2380	0	0	100	100
L–S., 1–50, D, 0, 1; 1–60, 2, 4; 1–75, 3, 3a, 3A	51	6902	15	11	7	32
Dudley (Sackett), No eradicant treatment						
No summer spray	3	3255	0	0	100	100
L–S., 1–50, D, 0, 1; 1–60, 2, 3, 3a, 3A	23	2733	7	2	1	10
L–S., 1–50, D, 0, 1; 1–60, 2, 3, 3A	9	2224	17	13	4	34
L–S., 1–50, 0, 1; 1–60, 2, 3, 3a, 3A	2	1476	8	5	1	14
L–S., 1–50, 1; 1–60, 2, 3, 3a, 3A	2	1626	8	5	1	14
L–S., 1–60, 2, 3, 3a, 3A	2	1281	28	22	13	62
Wealthy (Sackett), No eradicant treatment						
No summer spray	5	3215	0	0	100	100
L–S., 1–50, D, 0, 1; 1–60, 2, 3, 3a, 3A	9	2872	3	3	2	8
L–S., 1–50, D, 0, 1; 1–60, 2, 3, 3A	5	1739	5	5	2	12

[a] E.E. = Elgetol Extra (dosage expressed in per cent by volume); Cai = monocalcium arsenite (dosage expressed in per cent by weight); L–S = liquid lime-sulphur. Lead arsenate (2 to 3 lb. per 100 gal.) used in all summer sprays. Dates of spray application at Sackett orchard: D, May 4–5; 0, May 10–11; 1, May 18–19; 2, May 31–June 1; 3, June 14–15; 3a, June 24; 3A, July 6–7. At Stephenson orchard: Eradicant, April 22–23; D, May 5; 0, May 11; 1, May 19; 2, May 31; 3, June 17; 3A, June 29. At Goldman orchard: D, May 3; 0, May 9; 1, May 20; 2, June 2; 3, June 16; 3a, June 27; 3A, July 8; 4, Aug. 8–9.

[b] See footnote *b*, table 2.

Delicious in the ground-sprayed orchard than in the orchard that received no eradicant treatment; on July 11, 68.8 per cent fewer on McIntosh. On July 11, even where all pre-blossom sprays had been omitted in the ground-treated orchard, the leaf infection was fairly well controlled.

Results on fruit infection in the Stephenson and Samuelson orchards and certain other experimental orchards are shown in table 9. On June 20–21, about 3 weeks after petal-fall, trees that had received no summer spray showed 17 to 21 per cent of the fruit infected in the Stephenson orchard, as compared with 100 per cent in the stated orchards that received no eradicant spray. The average number of lesions per fruit for each McIntosh and Delicious in the Stephenson orchard was 98 per cent less than in the Samuelson orchard. It is noteworthy that the incidence of scab on nonsprayed fruit of Dudley and Wealthy in the Sackett orchard corresponded closely with that on McIntosh and Delicious in the Samuelson orchard. As was expected, scab developed severely in late season on the nonsprayed trees in the Stephenson orchard.

At harvest a sample of fruit was examined from each of the plots that received various summer spray programs in orchards respectively with and without eradicant fungicidal treatment. The scab on each fruit was classified as bad, commercial, or slight (see footnote *b,* Table 2). In the Stephenson and Samuelson orchards all the fruits were examined from each of a number of branches taken at random over each count tree. In the other orchards random samples were taken from each box as the fruit was picked.

The results appear in table 10. The eradicant fungicidal treatment in the Stephenson orchard so checked the epidemic (see also Tables 7, 8, and 9 and Fig. 6) that the disease was excellently controlled (1 per cent of fruits scabbed) by the 7-spray lime-sulphur program used, and fairly well controlled (15 per cent of fruits scabbed), even when the 3 prebloom treatments of this program were omitted. In the Goldman orchard, which received no eradicant treatment, an 8-spray lime-sulphur program failed to control the disease (32 per cent of fruit scabbed). A 6-spray lime-sulphur program on Dudley in the Stephenson orchard gave complete control of scab on the fruit, whereas a similar program in the Sackett orchard (no eradicant treatment) gave 34 per cent of the fruit scabbed. In the Sackett orchard, 62 per cent of the fruit was scabbed in the Dudley plot that received no pre-blossom spray, and lime-sulphur in the 4 treatments after blossom, whereas, in the Stephenson orchard, a similar 4-spray lime-sulphur program on McIntosh, on which scab is more difficult to control, gave only 15 per cent of fruit scabbed. It is noteworthy that, under the conditions encountered, omission of the delayed dormant and prepink sprays had comparatively little effect on scab control in either orchard.

The work in the Stephenson and Samuelson orchards was initiated as a preliminary orchard-scale experiment on eradicant fungicidal spraying, and was not designed for direct comparison with results from the Sackett and Goldman orchards. However, the data from the latter orchards seem so pertinent that they are presented with the notation that factors other than the spray treatments doubtless played some part in causing the differences in results in the several orchards.

A wholly satisfactory control on the effectiveness of the ground spray is difficult to attain. If a sufficient portion of an orchard of the size that was available for our work be left without treatment, the results may be seriously vitiated by air-drift of ascosporic inoculum from the nontreated to the treated portion. If a nearby orchard be used for the control, as the Samuelson orchard was in this work, some difference in conditions of the experimental orchards must be expected. Differences occur, however, in various parts of any orchard; and it is thought that the Stephenson and Samuelson orchards were as comparable as might have been parts of an orchard large enough to include them both. The data obtained by various methods on the reduction of the ascosporic inoculum by the treatment and on various aspects of disease development and control in the several orchards included in this study (Tables 6–10 and Figs. 5 and 6) all aid in the evaluation of the effects of the ground treatment, and thus contribute to the control of the experiment. It is noteworthy that these data, gained from many sources, are highly consistent. It is recognized, however, that they should be interpreted conservatively, pending further experimentation.

DISCUSSION

In the work reported herein attention was centered on two major aspects of the problem: (a) materials and methods for drastic suppression of the ascosporic inoculum of *Venturia inaequalis* by use of eradicant fungicides, (b) the effects of such reductions of the ascosporic inoculum on the epidemiology and control of the disease.

In the experiments performed in the two small orchards of Wealthy during the three seasons a copper-lime-arsenic mixture applied after harvest and before leaf-fall showed an indicated efficiency of 97 to 100 per cent in reducing the incidence of perithecia in the overwintered leaves of the treated orchard as compared with the nontreated. In the ground-spraying experiments in a commercial orchard Elgetol Extra, diluted 1–100 by volume in water, gave an indicated reduction of the effective ascosporic inoculum of about 96 per cent. Further work will be necessary to establish the relative merits of spraying after harvest and before leaf-fall and treating the dead leaves on the ground. However, the fact that the ground treatment largely avoids certain obstacles to fall spraying, especially spray injury, leads to increased interest in ground spraying.

The records on incidence of perithecia or discharged ascospores and of scab lesions, taken in the different experiments and by various methods, show clearly and very consistently that the reductions in the effective ascosporic inoculum resulted in drastic reductions in incidence of scab lesions. While the correspondence was closest in the data dealing with ascosporic infection, the relationship between numbers of perithecia or discharged ascospores and of primary lesions being approximately direct, the effects of the eradicant spraying were strikingly apparent throughout the season. The epidemics were much retarded and reduced. On the very susceptible late variety, McIntosh, a severe late development of scab occurred on nonsprayed trees in the ground-sprayed orchard, but the retardation and reduction seemed greatly to facilitate the effectiveness of summer spraying.

Much more work will be necessary before conclusions can be reached regarding the place eradicant fungicidal treatments of the type discussed herein may take in the control of this disease. Promising results have already been reported by other workers (1, 8). However, the full variability of epidemiological conditions has not yet been encountered, and the writers would regard it as unwise to relax the summer spray program in commercial-orchard practice until an adequate body of experimental data is accumulated to warrant such procedure. In the present stage of the study, the writers are centering attention on the attempt to develop a satisfactorily efficient control program. If this can be done, further consideration may then be given to possibilities of decreasing its cost.

It has been pointed out elsewhere (2, 7) that, despite its great accomplishments, protectant spraying still has some serious limitations. Besides permitting sufficient survival of the pathogen to provide an abundant annually recurring ascosporic inoculum, the protectant program for apple scab control is expensive, laborious, and uncertain of success under unfavorable conditions. Furthermore, materials that have sufficient fungicidal efficiency for scab control under conditions of severe occurrence of the disease are liable to cause objectionable injury to the foliage or fruit. One of the great difficulties, both in planning a suitable protectant program and in interpreting the results in relation to those from other programs, is the great variability in intensity of the epidemics encountered in different years or places and corresponding differences in the difficulty of control. In situations in which there is risk of severe occurrence of scab, a safe program ordinarily requires an amount of protection sufficient for success under the most severe conditions that may reasonably be anticipated. If eradicant spraying can be made to effect a sufficient retardation and reduction in the epidemic, it would seem possible that the less injurious materials, and perhaps fewer applications, could be employed in the protectant program. Furthermore, the chances of obtaining consistent results might be enhanced.

The experimental results herein reported, in confirmation and extension of those reported earlier (2, 6, 7), seem to establish for apple scab the principles (a) that the quantity or dosage of the primary inoculum is a major factor in determining the time and intensity of epidemic development, and (b) that the time and intensity of development of the epidemic are major factors in influencing the requirements for its control. They suggest the probability that these principles apply to many other plant diseases of this general type.

If ground spraying can be made economically and consistently to yield results of the type obtained last year, a reworking of summer spray programs in relation to the changed requirements for control incident to the eradicant treatment would seem to be indicated. Recommendations for application of the findings of this investigation to orchard practice are, therefore, withheld, pending the results of further studies now in progress.

SUMMARY

In 3 successive years a small Wealthy orchard was sprayed after harvest and before leaf-fall with a copper-lime-arsenic mixture designed to limit production of ascosporic inoculum of *Venturia inaequalis*. A similar nonsprayed orchard nearby served as a control. Neither orchard received any summer spray. Counts indicate a reduction by the spray of 97 to 100 per cent in incidence of fertile perithecia of *V. inaequalis* in the overwintered leaves and a closely corresponding reduction in ascosporic infection in the treated orchard, as compared with the nontreated. Data taken on the fruit at harvest indicate that the spray reduced incidence of lesions by 96, 91, and 98 per cent, respectively, in 1937, 1938, and 1939. No significant spray injury was observed on Wealthy, but some injury was at times observed on certain other varieties.

Small-scale treatments of overwintered leaves on the ground showed that Elgetol Extra and certain other materials are highly effective in limiting development of the ascosporic inoculum.

In 1939 the overwintered leaves on the ground in a 6-acre McIntosh orchard in sod were sprayed with Elegtol Extra, 1 per cent by volume in water, at the rate of 450 gallons per acre. A similar nontreated orchard .3 mile away served as a control. A few trees in these orchards received no summer spray. Ascospore discharge experiments and lesion counts indicated that the eradicant spray reduced the effective ascosporic inoculum by approximately 96 per cent, with closely corresponding reduction in ascosporic infection. At 3 weeks after petal-fall the scab epidemic on nonsprayed trees was reduced by about nine-tenths in the treated orchard, as compared with the nontreated. The retardation and reduction of the epidemic seemed greatly to facilitate control by the summer spray programs used.

The results reported establish for apple scab the principles (a) that the dosage of the primary inoculum is a major factor in determining the time and intensity of epidemic development and (b) that the time and intensity of development of the epidemic are major factors influencing the requirements for its control. They suggest the probability that these principles apply to many other plant diseases of this type. Recommendations for application of the findings of this investigation to orchard practice are withheld, pending the results of further studies now in progress.

LITERATURE CITED

1. BAINES, R. C. The effectiveness of eradicant sprays in control of apple scab in Indiana in 1939. Trans. Ind. Hort. Soc. 79 (1939): 74–77. 1940.

2. Keitt, G. W. Some problems and principles of orchard disease control with special reference to sanitation and related measures. Jour. Econ. Ent. 29: 43–52. 1936.
3. ———. Toxicity of the sodium salt of dinitro-o-cresol to *Venturia inaequalis*. Science (n.s.) 90: 139–140. 1939.
4. ———, and C. N. Clayton. Experiments with ground spraying for combating apple scab. Wis. Hort. 30: 246, 248. 1940.
5. ———, ———, and M. H. Langford. Eradicant fungicidal treatments in relation to apple-scab control. (Abstract) Phytopath. 30: 13. 1940.
6. ———, and L. K. Jones. Studies of the epidemiology and control of apple scab. Wis. Agr. Exp. Stat. Res. Bull. 73. 1926.
7. ———, and D. H. Palmiter. Potentialities of eradicant fungicides for combating apple scab and some other plant diseases. Jour. Agr. Res. [U. S.] 55: 397–438. 1937.
8. Palmiter, D. H., and J. M. Hamilton. Ground sprays as supplementary control for apple scab. Farm Res. [N. Y. State Sta.] 6(1): 1, 4. 1940.
9. ———, and G. W. Keitt. The toxicity of copper-lime-arsenic mixtures to certain phytopathogenic fungi grown on malt agar plates. Jour. Agr. Res. [U. S.] 55: 439–451. 1937.
10. Pastac, I. Les colorants nitrés et leurs applications particulières.[3] Journées de la Lutte Chimique contre les Ennemis des Cultures 38(4): [?]. 1937.
11. Truffaut, Georges, and I. Pastac. Chimiothérapie des maladies des plantes. Traitement d'hiver. Rev. de Chim. Indus. 41: 2–8; 34–39. 1932.

HOST RANGE OF PEACH–MOSAIC VIRUS IN WESTERN COLORADO[1]

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INTRODUCTION

The practice of eradicating peach trees in Colorado following manifestation of peach-mosaic symptoms has resulted since 1935 in a reduction in the incidence of peach mosaic. However, even with the present system of thorough inspection and complete removal of all diseased trees,[3] severe sporadic outbreaks have occurred yearly in localized areas. A possible reason for such outbreaks is that *Prunus* types other than the peach may harbor the virus of peach mosaic and may either manifest atypical symptoms or fail to show any symptoms. Another possibility is that naturally occurring mosaics, found on apricot, cherry, flowering peach, and plum, may show a relationship to peach mosaic. A further possibility is that certain horticultural varieties of peach may be slow in manifesting the symptoms of peach mosaic or may show atypical symptoms, thus harboring

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the causal virus and enabling it to be spread before the trees can be detected and removed.

The writers (2), in 1937, observed that the Maynard plum may be naturally infected with the peach-mosaic virus without expressing symptoms. Cochran and Hutchins (3), working in southern California, reported that when scions from almond, apricot, prune, plum, and Myrobolan plum, which were infected with the viruses of naturally occurring mosaics, were grafted on healthy J. H. Hale peach nursery trees, symptoms indistinguishable from those of peach mosaic were expressed in the peach leaves. Cochran and Hutchins (4) also found that when almond, apricot, plum, and prune nursery trees were inoculated with buds taken from peach trees affected with the peach-mosaic virus, they became symptomless carriers of the virus.

This paper presents the results of further studies on the host range of the peach-mosaic virus, the findings as to the infectious or non-infectious nature of naturally occurring mosaics on apricot, cherry, flowering peach, and plum, and the reactions of some horticultural varieties of the peach to the peach-mosaic virus.

HOST RANGE

In order to investigate the possible host range of the peach-mosaic virus, it was necessary to obtain healthy stock. Plants used in this study were as follows: almond (unknown variety), apricot (varieties Montgamet and Smith), cherry (variety Royal Duke), and plum (varieties Hungarian prune, Green Gage, Satsuma, Maynard, Blue Damson, and Wickson). Twenty-five 1-year-old nursery trees of each of the *Prunus* types and varieties just mentioned, with the exception of Royal Duke cherry, were tested to insure their freedom from the peach-mosaic virus. In the case of the Royal Duke cherry only 10 trees were used. In order to ascertain whether these plants were infected with a mosaic virus which might produce symptoms on peach, buds were inserted into healthy Elberta peach nursery trees. None of the Elberta trees so treated showed symptoms of mosaic.

Twenty nursery trees of each *Prunus* type and variety used, with the exception of the Royal Duke cherry, then were subjected to infection by the peach-mosaic virus by use of bud inoculations. In the case of the Royal Duke cherry only 5 trees were inoculated. The 5 remaining trees of each *Prunus* type and variety were held as controls. The buds used were obtained from Elberta trees showing typical symptoms of peach mosaic. These buds also were inserted into each of 20 Elberta trees and 20 seedling trees of the same variety that were free from the peach-mosaic virus. In September, 1936, all the inserted peach buds showed growth unions; the new leaves from these buds manifested the following spring characteristic symptoms of peach mosaic.

No variety tested (Table 1), with the exception of the Montgamet apricot, Hungarian prune, Elberta peach, and Elberta seedling peach, expressed mosaic symptoms the following spring after inoculation. In early spring, there appeared small, yellow flecked areas in the apricot leaves. Later in the growing season these disappeared, and larger yellow areas became evident. In addition faint ring spots also were observed. The leaves of the Hungarian prune showed distinct patterns of mosaic in early spring (Fig. 1, C), but, as the season progressed, these became less evident.

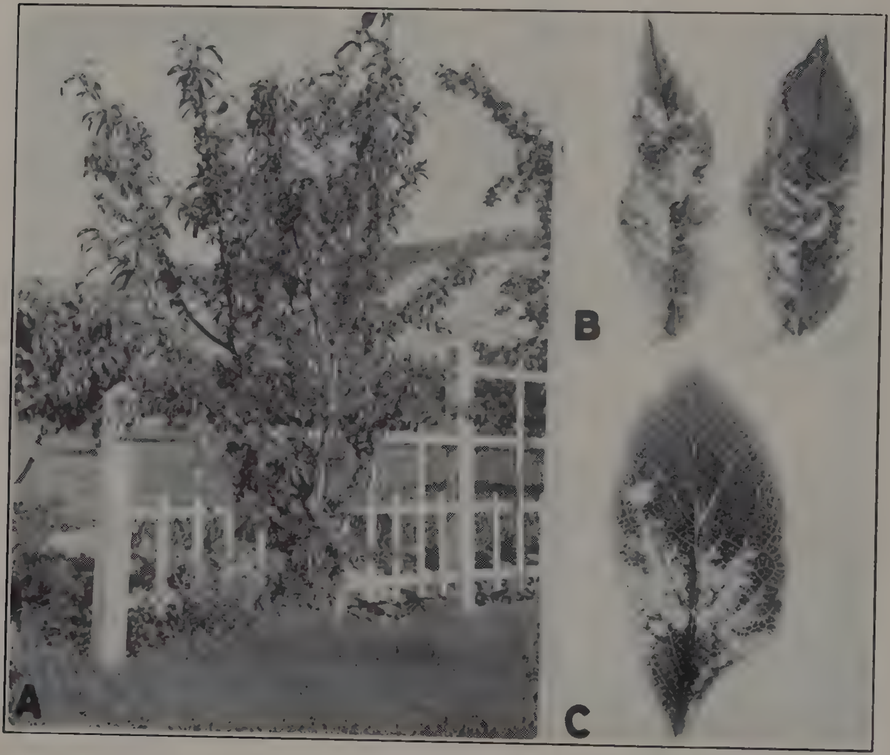

FIG. 1. Symptoms induced on *Prunus* types by the virus of peach mosaic. A. Infected flowering peach tree. The property on which this tree was found was adjacent to a peach orchard. A high percentage of the flowering peach trees in the Palisade area were found infected with peach-mosaic virus. B. Characteristic mosaic patterns on Elberta peach leaves from a plant inoculated by insertions of buds obtained from an infected Maynard plum tree. C. Mosaic pattern on a leaf from a Hungarian prune tree infected with the virus of peach mosaic.

In September, 1937, buds from all the previously mentioned horticultural varieties inoculated with the peach-mosaic virus (Table 1) were inserted into healthy Elberta trees and seedling nursery trees of the same variety. In all cases the inserted buds made growth unions, and symptoms of peach mosaic were manifested in the growth of the stock in the early spring of 1938. This indicated that the *Prunus* types used in this experiment, with the exceptions of peach, Montgamet apricot, and Hungarian prune, may, when inoculated, become infected with the peach-mosaic virus, although without expressing visible symptoms. This entire study was repeated in 1937, 1938, and 1939, giving essentially the same results as those listed in table 1. These results agree with those reported from similar studies by Cochran and Hutchins in southern California (4).

Because the majority of the *Prunus* types and varieties used in the previously described experiment, other than the peach, did not express mosaic symptoms, even though they were infected with the peach-mosaic virus, an experiment was conducted to determine whether such varieties were naturally infected with the peach-mosaic virus. It has been previously pointed out that such a condition was observed in the case of the Maynard plum (2). When buds taken from Maynard plum trees showing no symptoms of mosaic were inserted into healthy Elberta nursery trees in the fall, they showed severe mosaic symptoms the following spring (Fig. 1, B).

TABLE 1.—*The results of inoculating various Prunus types by bud insertions with the virus of peach mosaic*

Prunus types	Horticultural variety	Transmission of the peach-mosaic virus	Mosaic patterns in the leaves	Transmission back to the peach	
				Elberta seedling nursery trees	Elberta nursery trees
Apricot (*Prunus armeniaca*)	Montgamet	+	+	+	+
	Smith[b]	+	–	+	+
Almond (*P. communis*)	Unknown	+	–	+	+
Cherry[a] (*P. avium* × *P. cerasus*)	Royal Duke	+	–	+	+
Peach (*P. persica*)	Elberta	+	+	+	+
	Elberta seedling	+	+	+	+
Plum (*P. domestica*)	Hungarian prune	+	+	+	+
	Green Gage	+	–	+	+
(*P. salicina*)	Satsuma	+	–	+	+
	Maynard	+	–	+	+
(*P. insititia*)	Blue Damson	+	–	+	+
(*P. salicina* × *P. simonii*)	Wickson	+	–	+	+

[a] Since the buds of cherry inserted into peach tissue did not make permanent growth unions, they were inserted into Hungarian prune trees, which had been demonstrated to be infected with the peach-mosaic virus. Such inserted buds then made growth unions and produced satisfactory growth.

[b] This may be a local name for a standard variety.

Surveys of 200 commercial peach orchards and their environs revealed that trees of several varieties of *Prunus* types were growing in areas in which incidence of peach mosaic was quite high. In such localities trees of 3 varieties of apricot, 25 of plum, including 2 prune varieties, and a variety of almond were located, making a total of 1,340 plants. To ascertain whether these trees were carriers of the peach-mosaic virus, each plant was grafted with 2 healthy Elberta peach scions in the spring of 1938. During the growing season of 1938, scions on a majority of trees of each variety grafted made growth unions. In no case, however, did any of these developing scions express symptoms of peach mosaic. Representative grafted trees of each variety were again examined, and with like results, in the growing season of 1939.

NATURALLY OCCURRING MOSAICS ON PRUNUS TYPES OTHER THAN PEACH

Apparent mosaic symptoms have been observed frequently on apricot, flowering peach, cherry, and plum in and near orchards where many diseased peach trees had been removed. The question arose as to whether these symptoms resulted from infection by the peach-mosaic virus. In order to investigate this possibility, buds from 2 varieties of apricot (Montgamet and an unknown variety), cherry (variety Royal Duke), flowering peach, and plum (varieties Satsuma and Italian prune) showing such symptoms were inserted into 10 Elberta and 10 Elberta seedling peach nursery trees in September, 1936. The inserted buds in the majority of cases made growth unions, and the growth from these buds in the spring of 1937 revealed mosaic symptoms that in each case were characteristic of those shown by the parent trees. With the exception of those trees inoculated with buds from mosaic-affected flowering peach trees (Table 2), no symptoms of peach mosaic were apparent in the growth of either the Elberta trees or Elberta seedlings. In the former instance the mosaic patterns appearing in their leaves were characteristic of peach mosaic. Typical symptoms of the disease, such as petal breaking, leaf mottling, and internode shortening, resulting in tufting, were observed in the mosaic-affected flowering peach trees whence the buds were originally obtained (Fig. 1, A).

The naturally occurring mosaic found on the unknown variety of apricot was manifest in a mottling along and between the veins of the leaves. Such leaves frequently showed an abnormal crinkling (Fig. 2, D). The new stem growth of such diseased trees showed a distinct shortening of the internodes (Fig. 2, A). In addition, after the stone-hardening period, fruits became misshapen and exhibited a marked bumpiness (Fig. 2, E). The appearance of this mosaic was first observed on apricot growth that previously had been grafted to the Satsuma plum. Later, individual apricot trees showed the same symptoms. Satsuma plums, grafted with such apparently infected apricot scions, uniformly showed inconspicuous marginal mottling of the leaf blades. Only in a few instances did the fruits of such plums exhibit any noticeable raised areas. Satsuma plum trees, showing no apricot grafts, and exhibiting the previously described symptoms, were found later.

When buds from Satsuma plum trees and from trees of the unknown variety of apricot were inserted into Elberta seedling nursery trees in the fall of 1937, symptoms unlike peach mosaic appeared in the peach seedling growth the following spring (Table 2). Small ring spots were observed on some of the leaves, while others showed a marginal yellowing of the veins (Fig. 2, B). In addition still others exhibited small, irregular areas of primuline yellow (7). In the fall of 1938 buds from inoculated seedling peach, Sat-

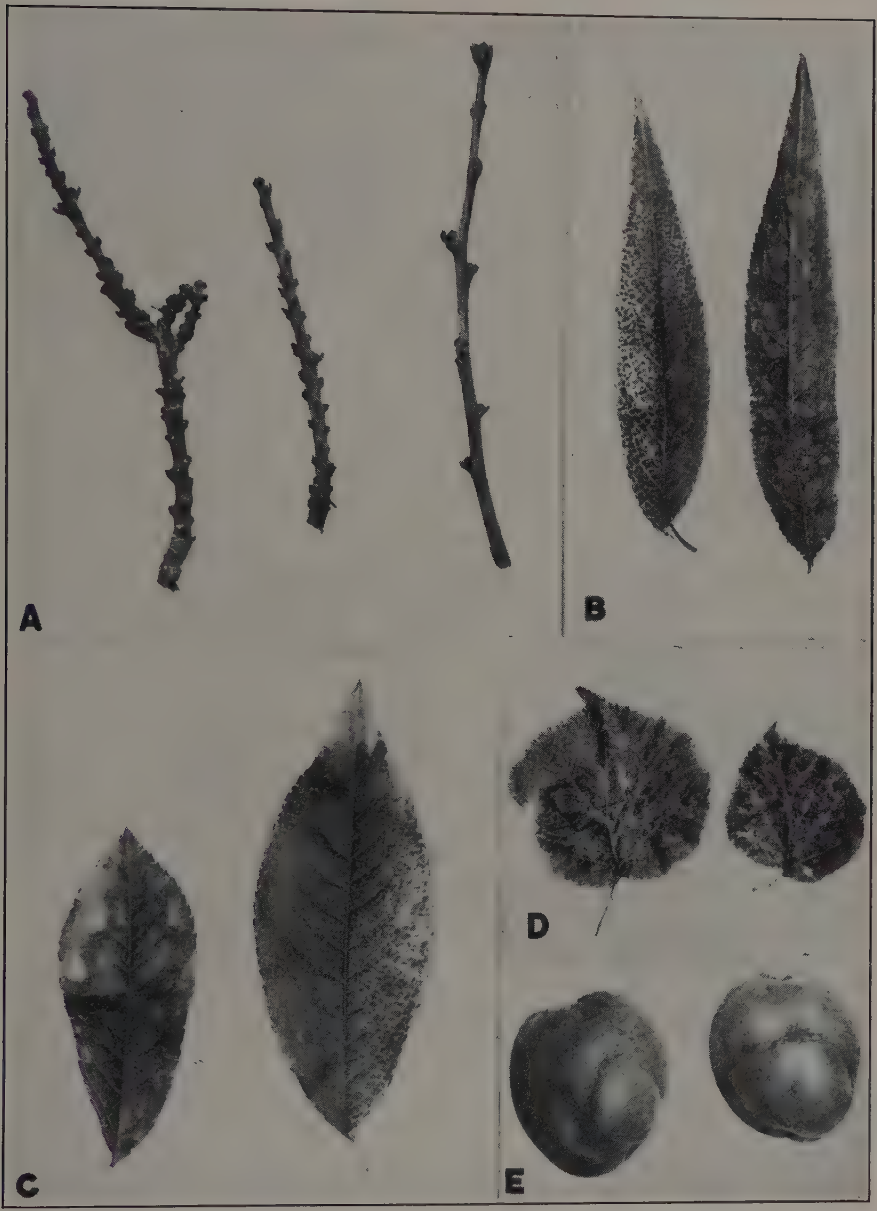

FIG. 2. The effect of the apricot-plum virus on apricot (unknown variety), seedling peach, and Elberta peach. A. Symptoms on new apricot stem growth. The two twigs on the left were taken from a diseased apricot tree, while the one on the right was taken from a healthy tree of the same variety for comparison. B. Symptoms on seedling peach leaves. C. Symptoms on Elberta peach leaves. D. Mottling of apricot leaves. E. Symptoms on apricot fruits.

suma plum, and the unknown variety of apricot were inserted into Elberta nursery trees. No symptoms were observed in the growth of the stocks during the season of 1939. In the spring of 1940, at the time of writing, however, the developing leaves of the Elberta peach stocks have shown symptoms that, with the exception of ring spots, were similar to those observed in the leaves of the previously discussed inoculated seedling-peach nursery trees (Fig. 2, C).

It was stated previously that the symptoms expressed in the leaf growth of the inserted buds of the various *Prunus* types studied were typical of those shown by each respective parent tree. In order to obtain further evidence as to the possible virus nature of these maladies, buds from Royal Duke cherry trees, Satsuma plum trees, and those of the unknown variety of apricot, all showing natural mosaics, were inserted into healthy nursery trees of the variety from which they were obtained in September, 1937 (Table 2). The following spring, mosaic symptoms characteristic of those shown by the parent trees from which the buds were obtained appeared both in the growth of the buds and in the inoculated trees. As shown in table 2, the viruses of the apricot and the plum mosaics were found interchangeably transmissible by bud inoculations, the symptom expressions indicating that these mosaics were caused by the same virus.

TABLE 2.—*The transmission of certain apparently naturally occurring mosaic viruses of Prunus types suspected of being related to the peach-mosaic virus*

Prunus types	Horticultural variety	Transmission				Symptoms on peach	
		To peach		To original host	To host other than peach	Peach mosaic type	Unlike peach mosaic
		Elberta	Elberta seedling				
Apricot (*Prunus armeniaca*)	Unknown	+	+	+	+[b]	–	+
	Montgamet	?[a]	?	–	...	–	–
Cherry (*P. avium* × *P. cerasus*)	Royal Duke	?	?	+	...	–	–
Peach (*P. persica*)	Flowering peach	+	+	+	...	+	–
Plum *P. salicina*	Satsuma	?	+	+	+[b]	–	+
Plum *P. domestica*	Italian Prune	?	?	...	...	–	–

[a] In such cases, as indicated by the absence of leaf symptoms, no transmission was evident. However, the possibility of transmission without symptoms to date cannot be excluded from consideration. At present, work is in progress designed to clarify this point.

[b] The virus affecting the unknown variety of apricot was transmissible to Satsuma plum and vice versa. The symptoms produced suggested that both mosaics were induced by the same virus.

Buds from Montgamet apricot trees, showing an apparently naturally occurring mosaic, were inserted into bearing trees of the same variety in September, 1938. In this particular disturbance the leaves showed mosaic patterns, and the fruit manifested ring spots at maturity. The majority of the inserted buds made growth unions, and the growth from these buds showed the same type of symptoms as were observed in the trees whence the buds were originally obtained. No such symptoms were manifested in the growth of the grafted trees in the season of 1939. In like fashion buds from the Italian prune trees showing an apparent mosaic were inserted into trees of the same variety. As was observed in the case of the grafted Montgamet apricot trees, symptoms were expressed in the growth from the inserted buds but not in that of the stock trees.

The previously described studies indicate the existence of mosaics occurring on varieties of apricot, cherry, and plum in western Colorado. Such mosaics apparently have no relationship to peach mosaic. In addition, the questionable disturbances observed in the Montgamet apricot and the Italian prune likewise have no bearing on the peach-mosaic problem.

THE REACTIONS OF PEACH VARIETIES TO THE PEACH-MOSAIC VIRUS

Since the beginning of the peach-mosaic investigations in Colorado, symptom manifestations of the disease have been recorded (1, 5). Similar recordings have been made in Texas and California (5). Observations to date indicate that all peach varieties in Colorado are susceptible to infection by the peach-mosaic virus, although the symptom manifestation seems to vary with the different varieties. In some varieties symptoms of peach mosaic may not be detected in all seasons of the year, yet no variety is known to harbor the virus without showing symptoms at some time (5). In the Carman, Guinn, Salwey, Wonderful, and Victor varieties of peach there is some evidence to the effect that the elapse of a longer period of time is necessary to bring about the expression of recognizable symptoms than that required for a similar expression in the Elberta and J. H. Hale varieties.

It has been observed that the disease is easily recognized in orchards planted only to the Elberta and J. H. Hale varieties, and that the removal of infected trees in early spring and throughout the growing season definitely arrests the spread of peach mosaic in these orchards (Table 3, orchards No. 1, 2, and 3). In some orchards, however, where Elberta, J. H. Hale, Carman, Salwey, Guinn, Phillips Cling, and other varieties were grown together, the spread of the disease was not arrested following such persistent eradication (Table 3, orchards No. 4, 5, 6, and 7).

In field studies in the spring of 1938, 200 suspected peach trees, consisting of the varieties Carman, Guinn, Salwey, Wonderful, and Victor, were indexed by grafting them with healthy Elberta scions. These scions were obtained from trees growing in a locality where the peach-mosaic disease has not been observed to date and were tested to insure their freedom from the peach-mosaic virus. The growth of the Elberta test-scions frequently expressed symptoms of peach mosaic (Fig. 3, left), while the tree on which the graft was made showed no apparent symptoms. In other cases the tree showed only slight to moderate symptoms, while the scion showed moderate to severe symptoms. In still other instances symptoms in the scion were lacking, while the grafted tree expressed only slight symptoms. Finally, in numerous cases, symptoms were expressed in neither the scion (Fig. 3, right) nor stock. In several instances Elberta scions, grafted

TABLE 3.—*Comparison of the incidence of peach mosaic from 1935–1939, inclusive, in orchards composed of only the Elberta and J. H. Hale varieties with the incidence in orchards of mixed varieties following continued eradication*

Orchard	Varieties	1935		1936		1937		1938		1939	
		Total number trees in orchard	Number diseased trees removed	Total[a] number trees in orchard	Number diseased trees removed	Total number trees in orchard	Number diseased trees removed	Total number trees in orchard	Number diseased trees removed	Total number trees in orchard	Number diseased trees removed
1	Elberta	1196	317	875	64	811	10	801	2	799	11
2	Elberta	589	251	341	47	294	3	291	5	286	3
3	Elberta and J. H. Hale	817	138	583	39	647	1	646	8	638	6
4	Elberta, Carman, and Salwey	1047	52	995	30	965	64	901	140	761	69
5	Elberta, Phillips Cling, and Carman	3699	275	3424	143	3281	50	3031	134	2997	46
6	Elberta, Phillips Cling, and Carman	701	24	677	10	667	47	620	209	411	120
7	Elberta, Guinn, J. H. Hale, Victor, Salwey, Carman, Wonderful, Phillips Cling, etc.	2383	190	2192	86	2106	119	1987	632	1355	304

[a] The figures shown in the above table do not include trees that are transplanted to replace those lost by eradication. Transplantings were begun in 1936 and were continued until the present time. To the best of the writers' knowledge, none of the transplants in the above-listed orchards have shown symptoms of peach mosaic to date. Further observations have revealed that throughout the entire peach-growing area in western Colorado only a very few trees transplanted since the beginning of the investigations showed evidence of natural infection by the peach-mosaic virus.

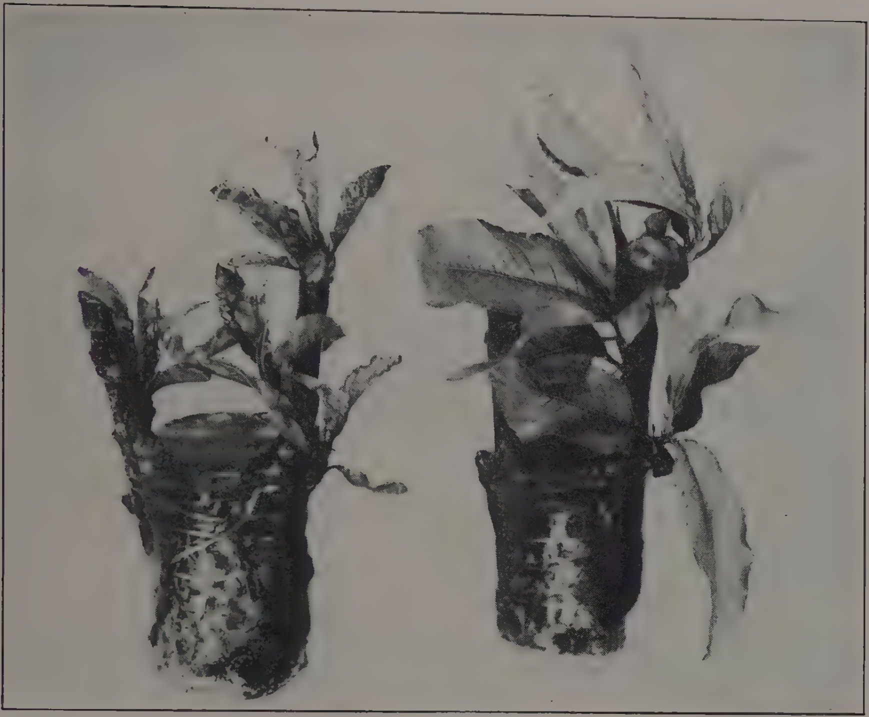

FIG. 3. Method of indexing trees of certain peach varieties suspected of harboring the virus of peach mosaic. Left, Elberta scions growing on Carman stock. Symptoms of peach mosaic are evident in the leaves of the scions. Right, an unaffected Elberta scion on a second Carman stock. All scions were obtained from Elberta peach trees known to be free from the virus of peach mosaic. The Carman stock trees showed no symptoms of peach mosaic.

to trees of the Carman, Salwey, Wonderful, and Victor varieties, showed slight to moderate symptoms of peach mosaic in the springs of 1938 and 1939. No recognizable leaf symptoms of peach mosaic were observed in the stock trees during this period.

The existence of more than one strain of the peach-mosaic virus has been indicated in western Colorado since 1935. Evidence of this nature recently has been recorded by Hutchins and Cochran in southern California (6). The existence of strains of the virus may be an explanation for the results obtained in the study of the reactions of peach varieties to the peach-mosaic virus.

SUMMARY

Trees of an unknown variety of almond, Montgamet and Smith apricots, Royal Duke cherry, and Hungarian prune, Green Gage, Satsuma, Maynard, Blue Damson, and Wickson plums were subjected to infection by the peach-mosaic virus through bud inoculations. Of the trees thus treated, only the Hungarian prune and Montgamet apricot showed mosaic symptoms. Trees of all the varieties inoculated, whether expressing symptoms or not, were, however, shown to have become infected by the virus of peach mosaic. This infection was detected by virtue of bud insertions into healthy Elberta trees and Elberta seedling peach nursery trees.

Grafting a total of 1,340 plum, apricot, and almond trees with two healthy Elberta peach scions per tree showed no tree infected with the peach mosaic virus. These trees included 25 varieties of plum, 3 of apricot, and 1 variety of almond.

Naturally occurring mosaics have been observed on apricot (varieties Montgamet and unknown), Royal Duke cherry, flowering peach, and plum (varieties Satsuma and Italian prune). In order to determine whether these mosaics were the result of infection by the virus of peach mosaic, buds from trees of each variety showing a mosaic were inserted into healthy Elberta trees and Elberta seedling peach nursery trees. Only trees inoculated with buds from the affected flowering peach trees showed peach-mosaic symptoms. Mosaic symptoms unlike those of peach mosaic appeared in the growth of the Elberta seedling and Elberta peach nursery trees inoculated with buds obtained from the affected unknown variety of apricot and affected Satsuma plum. None of the peach trees grafted with buds obtained from trees of varieties showing the other naturally occurring mosaics expressed any symptoms. The virus causing the mosaic of the unknown variety of apricot was found to be transmissible to the Satsuma plum, and *vice versa.* The symptoms produced suggested that both mosaics were induced by the same virus. By bud inoculations of the same variety from which the buds were obtained, the virus natures of all naturally occurring mosaics studied, with the exceptions of those found on the Italian prune and Montgamet apricot, were established.

A five-year study of the incidence of the peach-mosaic disease in 7 orchards showed that the eradication of diseased trees definitely arrested the spread of mosaic in 3 orchards planted only to the Elberta or J. H. Hale or both varieties. However, in four orchards of mixed plantings in which varieties such as the Carman, Salwey, Guinn, Victor, J. H. Hale, Elberta, and Phillips Cling were growing together, the spread of peach mosaic was not arrested by persistent eradication.

Grafting Elberta test-scions on 200 peach trees of the varieties Carman, Guinn, Salwey, Wonderful, and Victor gave variable results. These trees, some of which were suspected of harboring the peach-mosaic virus, were selected at random. In many cases where no mosaic symptoms were evident in the stocks, slight to moderate symptoms were expressed in the growth of the scions. In other instances where slight symptoms were observed in the stocks, moderate to severe symptoms were expressed in the growth of the scions. In still other cases where slight symptoms were apparent in the stocks, no symptoms were expressed in the growth of the scions. Finally, numerous trees showed symptoms in neither the stock nor scion growth.

LITERATURE CITED

1. BODINE, E. W. Peach mosaic disease in Colorado. Colo. Agr. Exp. Stat. Bull. 421. 1936.

2. ———, and L. W. DURRELL. The Maynard plum—a carrier of the peach mosaic virus. Science (n. s.) 86: 81. 1937.
3. COCHRAN, L. C., and LEE M. HUTCHINS. Peach-mosaic host-relationship studies in southern California. (Abstract) Phytopath. 27: 954. 1937.
4. ———, and ———. Further studies on host-relationships of peach mosaic in southern California. Phytopath. 28: 890–892. 1938.
5. HUTCHINS, LEE M., E. W. BODINE, and H. H. THORNBERRY. Peach mosaic, its identification and control. U. S. Dept. of Agr. Circ. 427. 1937.
6. ———, and L. C. COCHRAN. Peach-mosaic virus strain studies. (Abstract) Phytopath. 30: 11. 1940.
7. RIDGWAY, R. Color standards and color nomenclature. 43 pp. Illus. Washington, D. C. 1912.

VARIATION IN THE GERMINATION OF CHLAMYDOSPORES OF USTILAGO ZEAE[1]

M. F. KERNKAMP AND M. A. PETTY

(Accepted for publication September 10, 1940)

In the course of experimental work on the variability of *Ustilago zeae* (Beck.) Ung. at University Farm, St. Paul, Minnesota, the germination of a large number of chlamydospores from many crosses and collections has been observed. Frequent deviation from the classical 4-cell promycelial type was conspicuous, and it appeared that certain types of promycelia were characteristic for given crosses and collections.

Previous results have shown that lysis of promycelia (2) and the tendency toward production of sporidia or hyphal branches on the promycelia (4) are heritable characters. Other workers (1, 3, 5) have described the details of germination and have illustrated several types of promycelia, but extensive experiments to determine whether characteristic types of germination are attributable to genetic factors have not been recorded.

Therefore, the writers undertook to determine the range of germination types in several crosses and collections of *Ustilago zeae* and to find out whether there were indications that the types are governed by genetic factors.

EXPERIMENTAL PROCEDURE AND RESULTS

Chlamydospores from the sources indicated in table 1 were germinated on potato-dextrose agar smears on cover slips inverted over van Tieghem cells in Petri dishes. The Petri dishes, without covers, were placed in a large moist chamber, making it possible to germinate spores from several crosses and collections at the same time and under the same conditions.

One hundred or 200 promycelia of chlamydospores from each cross or collection were studied and records made of the number of cells in the promycelia, the number of sporidia on each cell, the number of hyphal branches on each promycelium, the number of chlamydospores with 2 opposite promycelia, the number of chlamydospores with sporidia formed directly on them, and the number of lysing promycelia.

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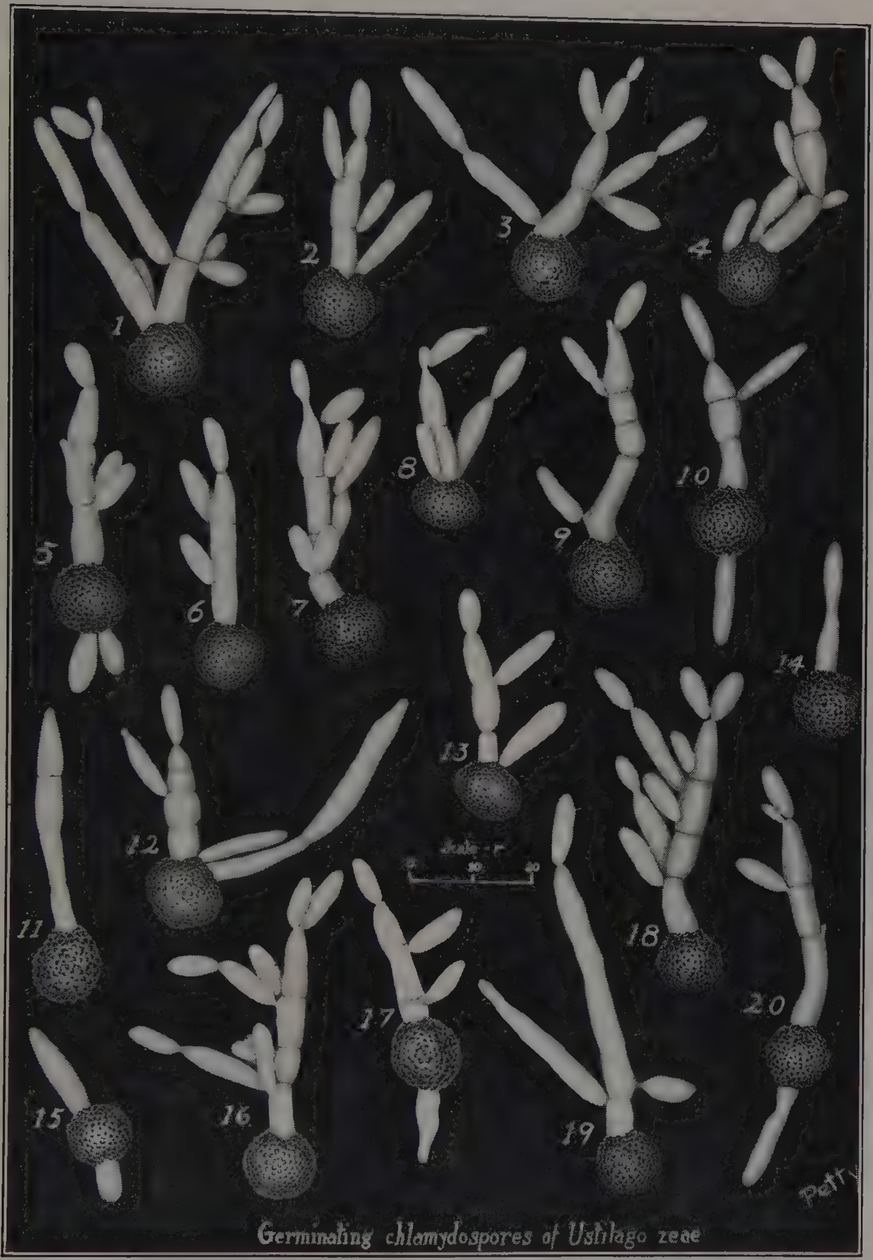
TABLE 1.—*Source of chlamydospores of Ustilago zeae*

Name of cross or collection	Parentage	Where and from whom obtained
Cross 60	$51D_1 \times 51G_2$	Writers
Cross 80	$70B_1 \times 70G_2$	Do
$60A_1 \times 60A_3$	$60A_1 \times 60A_3$	Do
$Arg.Y_2 \times Arg.H_3$	$Arg.Y_2 \times Arg.H_3$	Do
Solopathogen $72L_e$	Solopathogenic line $72L_e$	Do
Solopathogen $72M_f$	Solopathogenic line $72M_f$	Do
Hastings	....	Collected by writers, Hastings, Minn.
Brooklyn Center	....	Collected by writers, Brooklyn Center, Minn.
Wisconsin	....	Collected by D. H. Bowman, Madison, Wisconsin
Louisiana	....	Collected by K. W. Kreitlow, Baton Rouge, Louisiana
Field U. F. No. 1	....	Collected by writers, University Farm, St. Paul, Minn.
Field U. F. No. 2	....	Do
Argentina No. 1	....	Collected by Dr. José Vallega, Argentina
Argentina No. 2	....	Do

Figures 1 and 2 are camera-lucida drawings representing a random sample of some of the different types of germinating chlamydospores encountered. Among these are 1–8-cell promycelia with none to several sporidia or hyphal branches on each cell. Some germ tubes never produce any sporidia but grow directly into indeterminate hyphae (Fig. 2, number 5). Many other variations occur, but the writers want particularly to call attention to the predominant and characteristic types.

The classical 4-cell promycelium is represented by numbers 16 and 18 in figure 1. Other predominant types are: 3-cell promycelia (Fig. 1, numbers 2, 4, and 6, and Fig. 2, numbers 2, 7, and 9); sporidia formed directly on the chlamydospores (Fig. 1, numbers 1, 2, 4, 8, and 13, and Fig. 2, numbers 2, 3, 7, and 11); promycelia with hyphal branches (Fig. 1, numbers 12, and 19, and Fig. 2, numbers 6, 8, and 10); chlamydospores with 2 opposite promycelia (Fig. 1, numbers 10, 15, 17, and 20); and promycelia showing lysis.

The frequency of germination types in individual crosses or collections is given in tables 2 and 3. It is evident that there is an extreme range of types in different crosses and collections, but one type tends to predominate within a single cross or collection. For example, the classical 4-cell type varies from 11 to 78.5 per cent, and the percentage of chlamydospores with 2 opposite promycelia ranges from 2 to 60 in different spore lots (Table 3). Comparison of the prevalence of all of the predominant types between and within all of the crosses and collections is made in table 3. These percentages do not total 100, since there are some chlamydospores that fall in more than one group, and others are not included in any of these groups. For example, figure 2, number 8, has a 4-cell promycelium and hyphal branches, and figure 2, number 1, has a sporidium arising from the chlam-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Germinating chlamydospores of Ustilago zeae
Petty

FIG. 1. Camera-lucida drawings of germinating chlamydospores of *Ustilago zeae,* showing range of types.

ydospore, as well as a germ tube on the opposite side from the promycelium.

From tables 2 and 3 it is clear that one type tended to predominate in each of the crosses and collections studied. As the differences occurred, even though all spores germinated in the same environment, it appears that

TABLE 2.—*The percentages of various types of promycelia formed on germinating chlamydospores of different crosses and collections of Ustilago zeae*

Kind of germination	Cross or collection													
	Cross		60 A_1 × 60 A_3	Arg.Y_2 × Arg.H_3	Solopath.		Hastings	Brooklyn Center	Wis.	La.	Field U. F.		Argentina	
	80	60			72L_e	72M_f					No. 1	No. 2	No. 1	No. 2
1-cell promycelia	......	......	1	76.00	......	......	2	......	......	......	......	......	1	1
2-cell promycelia	0.5	2	5	......	......	5	6	1	0.5	......	......	2	1	5
3-cell promycelia	20.5	21	26	0.66	6	9	20	8	2.0	5.0	2	36	11	11
4-cell promycelia	38.5	14	17	6.00	13	32	26	11	78.5	50.0	18	18	36	45
5-cell promycelia	......	......	......	4.66	11	4	2	......	1.5	3.5	......	4	4	6
6-cell promycelia	......	......	......	......	4	2	......	......	0.5	0.5	......	3	1	2
7-cell promycelia	......	......	......	1.33	......	1	......	1	......	......	......	......	......	2
8- or more-cell promycelia	0.5	......	......	......	......	......	......	......	......	0.5	......	1	......	......
1-cell promycelia with sporidia on chlamydospore	......	......	......	......	1	1	......	2	......	......	......	1	......	1
2-cell promycelia with sporidia on chlamydospore	2.5	5	6	......	3	3	8	13	0.5	1.5	......	3	2	2
3-cell promycelia with sporidia on chlamydospore	35.5	25	37	63.00	30	24	32	18	10.5	24.5	20	15	35	11
4-cell promycelia with sporidia on chlamydospore	......	......	......	4.66	8	5	2	......	1.0	2.5	......	7	4	2
5-cell promycelia with sporidia on chlamydospore	......	......	......	......	5	4	......	......	......	0.5	......	2	1	1
6-cell promycelia with sporidia on chlamydospore	......	......	......	......	......	......	......	......	......	......	......	......	......	1
7-cell promycelia with sporidia on chlamydospore	......	......	......	......	......	......	......	......	......	......	......	......	......	1

TABLE 2.—(*Continued*)

Kind of germination	Cross or collection													
	Cross		$60\ A_1 \times 60\ A_3$	$Arg.Y_2 \times Arg.H_3$	Solopath.		Hastings	Brooklyn Center	Wis.	La.	Field U. F.		Argentina	
	80	60			$72L_e$	$72M_f$					No. 1	No. 2	No. 1	No. 2
1-cell promycelia with a promycelium on opposite side	....	......	3	....	......	......	......	1	....	....	......	......	......	......
2-cell promycelia with a promycelium on opposite side	0.5	......	1	....	2	......	......	13	1.0	....	6	2	1	1
3-cell promycelia with a promycelium on opposite side	1.0	27	2	....	11	5	......	15	3.5	8.0	38	6	2	4
4-cell promycelia with a promycelium on opposite side	0.5	4	2	0.66	5	4	......	2	0.5	2.0	12	......	1	2
5-cell promycelia with a promycelium on opposite side	....	......	......	....	......	......	......	......	....	1.0	2	......	......	1
6-cell promycelia with a promycelium on opposite side	....	......	......	....	......	......	......	1	....	....	......	......	......	......
1-cell promycelia with sporidia on chlamydospore and opposite promycelium	....	......	......	....	......	......	......	1	....	....	......	......	......	......
2-cell promycelia with sporidia on chlamydospore and opposite promycelium	....	1	......	....	......	......	......	10	....	....	2	......	......	1
3-cell promycelia with sporidia on chlamydospore and opposite promycelium	....	......	......	....	1	1	2	2	....	0.5	......	......	......	......
4-cell promycelia with sporidia on chlamydospore and opposite promycelium	....	1	....	....	......	......	......	1	....	....	......	......	......	......

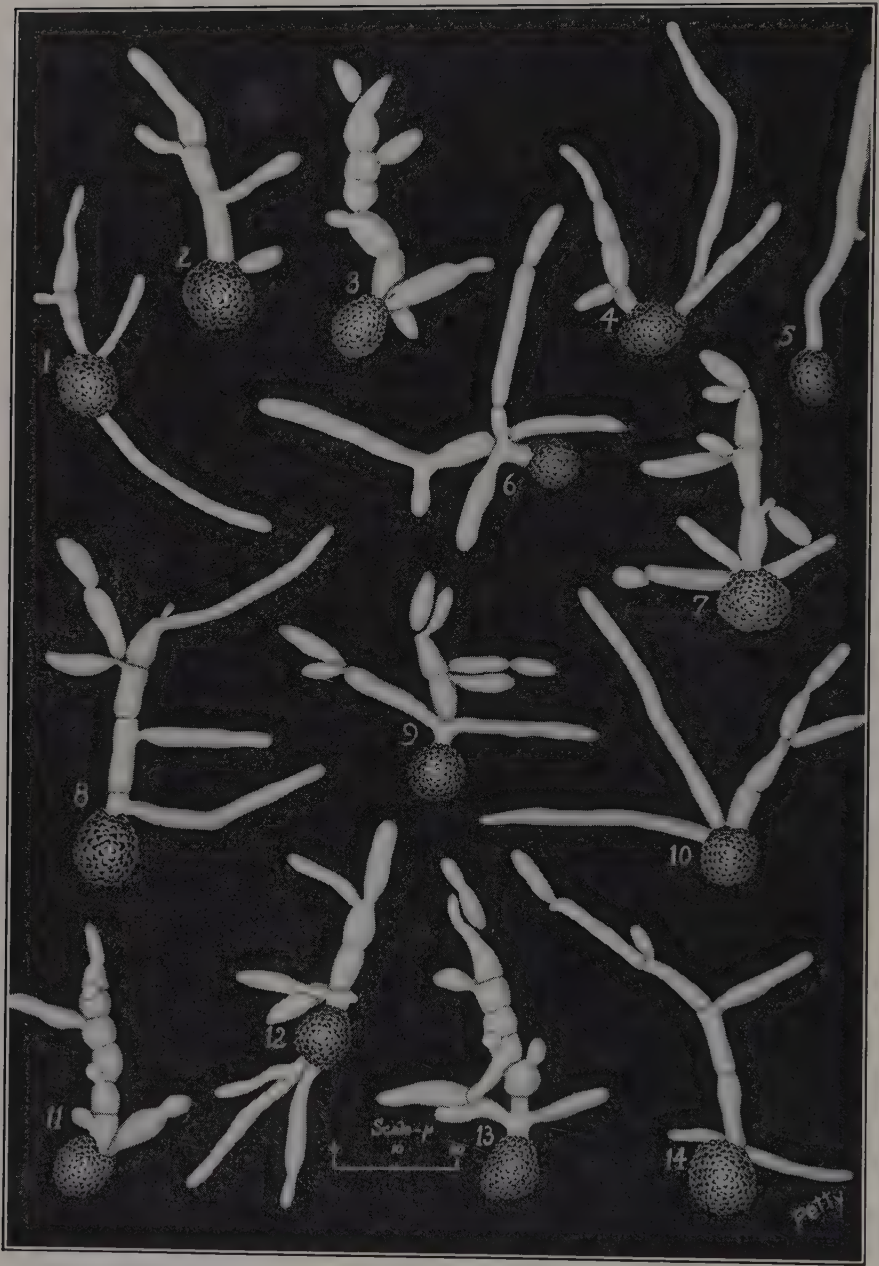

FIG. 2. Camera-lucida drawings of germinating chlamydospores of *Ustilago zeae*, illustrating particularly deviations from the supposedly normal 4-cell promycelium.

all of the major germination types are governed by genetic factors. This conclusion is supported by the fact that it already has been shown that tendency to lyse and for promycelia to produce sporidia or hyphal branches is governed by genetic factors (2, 4).

TABLE 3.—*The predominant germination types and percentages*[a] *of each in 14 crosses and collections of Ustilago zeae*

Cross or collection	Percentage prevalence of types indicated				
	4-cell promycelia	Sporidia borne directly on chlamydospores	Chlamydospores with two opposite promycelia	Promycelia with one or more hyphal branches	Lysis
Louisiana	50.0	29.5	11.50	18.0	0
Wisconsin	78.5	12.0	5.00	20.0	0
Hastings	26.0	44.0	2.00	0.0	0
Argentina No. 1	36.0	42.0	4.00	25.0	0
$60A_1 \times 60A_3$	17.0	43.0	8.00	16.0	0
Brooklyn Center	11.0	47.0	46.00	4.0	0
Cross 60	14.0	30.0	33.00	17.0	0
Field U. F. No. 1	18.0	22.0	62.00	1.5	0
Cross 80	33.5	33.5	2.00	43.0	0
Solopathogen $72L_e$	13.0	48.0	19.00	70.0	0
Solopathogen $72M_f$	32.0	38.0	10.00	82.0	0
Argentina No. 2	45.0	20.0	9.00	84.0	0
Field U. F. No. 2	18.0	28.0	8.00	44.0	5
Arg. $Y_2 \times$ Arg. H_3	25.0	10.6	0.66	0.0	76

[a] Percentages do not total 100, since some chlamydospores fall in two groups.

These results clearly demonstrate that the production of the classical 4-cell promycelium is by no means characteristic for *Ustilago zeae* in general, but any one of several types may be normal for a given cross or collection. In the crosses and collections studied here, only 5 types predominated; but the writers see no reason why any one of the other types could not be found to be most prevalent, if more collections or crosses are studied.

SUMMARY

The writers studied the type of germination of chlamydospores from 8 field collections, 4 crosses between monosporidial lines, and 2 monosporidial diploid lines of *Ustilago zeae.*

Twenty-five germination types were observed; of these, the production of the supposedly normal 4-cell promycelium, with a sporidium on each cell, was neither the most characteristic nor prevalent.

There was a tendency for a given germination type to predominate within each cross or collection, although there was considerable variation with crosses and collections.

The 5 most prevalent germination types and the number of crosses or collections in which each predominated follow: Promycelia bearing one or more hyphal branches in place of sporidia, 5; Chlamydospores functioning as basal promycelial cells, thus forming sporidia directly on the spore, 4; Chlamydospores with 2 opposite promycelia, 2; 4-cell promycelia, 2; lysing promycelia, 1.

It appears that the tendency toward a given germination type is determined by genetic factors.

LITERATURE CITED

1. BREFELD, OSCAR. Botanische Untersuchungen über Hefenpilze. Heft V. Brandpilze L. 1883.
2. CHILTON, ST. JOHN P. The occurrence of lysis in certain crosses of *Ustilago zeae*. (Abstract) Phytopath. 28: 5. 1938.
3. HITCHCOCK, A. S., and J. B. S. NORTON. Corn smut. Kan. Agr. Exp. Stat. Bull. 62. 1896.
4. KERNKAMP, M. F. Genetic and environmental factors affecting growth types of *Ustilago zeae*. Phytopath. 29: 473–484. 1939.
5. STAKMAN, E. C. Spore germination of cereal smuts. Minn. Agr. Exp. Stat. Bull. 133. 1913.

STUDIES ON THE NATURE OF SPINDLING SPROUT OF POTATO[1]

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(Accepted for publication August 19, 1940)

Hawaii annually imports large quantities of seed potatoes for winter production. In the fall of 1937, several shipments of potatoes, received from mainland United States, particularly North Dakota, contained appreciable numbers of tubers that showed typical "spindling-sprout" or "hair-sprout." Instead of possessing 2 to 3 vigorous, sturdy sprouts, these tubers were characteristically multisprouted, the sprouts being very slender, spindly, and threadlike. Fields planted with seed showing this condition in a fairly high percentage yielded so low as to fail to pay for the cost of planting.

In this paper are to be found data on yield, number of sprouts per plant, and type of plant growth obtained when normal tubers are grafted with cores from spindling-sprout tubers and when spindling-sprout tubers are grafted with cores from normal tubers. Chemical analyses of normal and diseased tubers are also presented.

LITERATURE REVIEW

Relatively little exact information as to the cause of spindling sprout rewards a search of the literature. The condition has been ascribed to potato leaf-roll virus by Gilbert (1), by Morris and Young (4) to witches'-broom virus, while Stewart and Sirrine (7) suspected that the disease was caused by abnormal environmental conditions, particularly excessive temperature or drought. Werner (10), however, could detect no effect of temperature or soil moisture on the productivity of seed potatoes grown at controlled temperatures, with soil moisture varied. His highest temperature was only 85° F.

Leach (3) has recently summarized our knowledge of spindling sprout. He adds Fusarium wilt as a suggested cause, but states "it is possible that the condition may be caused by either of several different factors. Whatever the cause may be it is not definitely known."

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[1] Published with the approval of the Director as Technical Paper No. 44 of the Hawaii Agricultural Experiment Station.

MATERIALS AND METHODS

Grafting

Spindling-sprout tubers and normal tubers were selected from several bags of Bliss Triumph seed received in Honolulu in December, 1937. Specimens of both types are shown in figure 1, A. Grafts were made by removing, with a cork borer, a core from one tuber and replacing it with a core from another. The following vegetative unions were successfully made: A. Core, buds absent, from spindling-sprout tuber, inserted into normal tuber. B. Core, buds present, one at each end, from normal tuber, inserted into spindling-sprout tuber.

Two hundred tubers of each type were grafted. They were planted in the field, 4 rows of each, with 50 tubers in each row, 1 ft. apart in the row, to give 4 replications per treatment. At the same time, 200 normal tubers and 200 spindling-sprout tubers were similarly planted to give a total of 16 plots. The land received a preplanting fertilization with a 15–30–6 formula, at a rate of approximately 4 lb. per row, or about 1160 lb. per acre.

Chemical Analyses

Analyses were made of spindling-sprout and normal tubers from the same bag. Twenty-five tubers of each class were selected and duplicate 50-g. samples extracted with 80 per cent alcohol and made up to a final volume of 500 cc. The remaining residue was transferred to a weighed beaker and dried in a vacuum oven at 80° C. The dry residue was then ground in an iron mortar to pass a 40-mesh sieve. Methods of analysis are described below:

Solids. Total soluble solids were determined on duplicate 50-cc. aliquots of the extract.

Nitrogen. Total soluble nitrogen was determined as described by Jones (2).

Partition of Nitrogen. The alcohol was removed from 200 cc. of the extract, transferred to a 50-cc. volumetric flask, and cleared with magnesium sulphate and chloroform. Ammonia and amide nitrogen were determined on 5-cc. aliquots of the cleared extract, using the method described by Stuart (9). Amino nitrogen was determined on 2-cc. aliquots of the cleared extract with the micro-Van Slyke apparatus.

Sugars. An aliquot of the alcoholic extract was freed of alcohol and cleared with neutral lead acetate and potassium oxalate. Reducing sugar was determined on an aliquot of the cleared extract by an iodine method (7). Sucrose was found by hydrolyzing an aliquot of the extract with invertase solution and then determining the increased reducing power by the method used for reducing sugar.

Insoluble Residue. Insoluble nitrogen was obtained by the micro-Kjeldahl method of Pregl (5). The reducing power of 1-g. samples of the residue, refluxed with 2.5 per cent HCl for 2½ hr. determined the acid-hydrolyzable polysaccharides present.

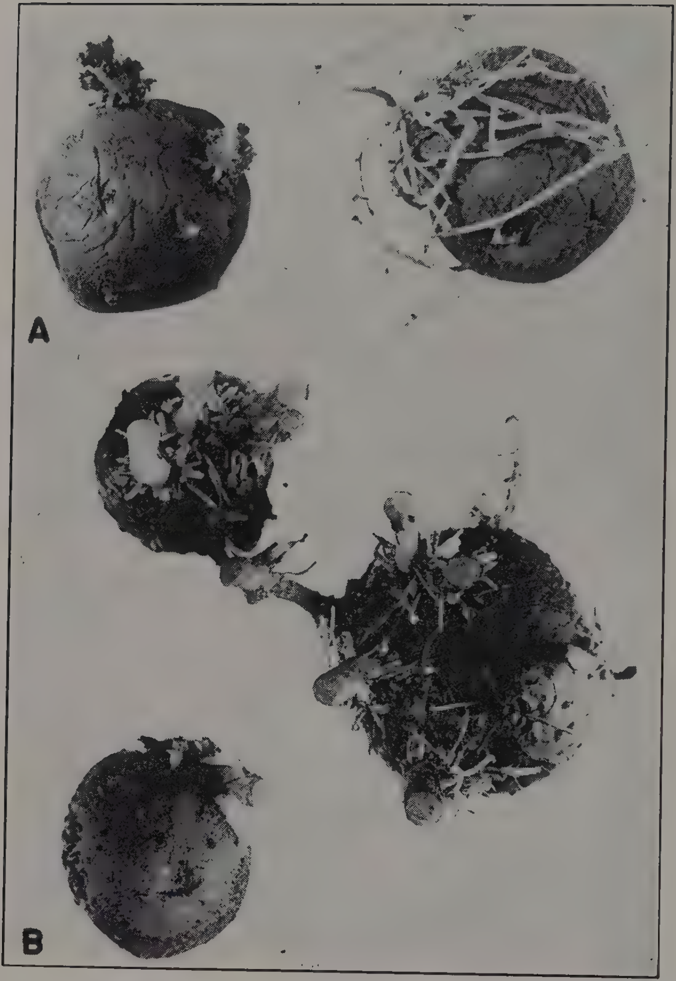

FIG. 1. A. Spindling-sprout tuber (right) and normal tuber, variety Bliss Triumph. B. Effect on sprout development of grafting a core taken from a spindling-sprout tuber into a normal tuber. Lower left, ungrafted tuber.

RESULTS AND DISCUSSION

Grafting

Plants produced by normal tubers, plug-grafted with spindling-sprout cores, emerged from the soil 1–2 days earlier than plants from normal nongrafted tubers and produced a significantly greater number of sprouts per hill. In size of leaf and size and shape of stems, shoots from grafted plants compared favorably with shoots from nongrafted plants. The effect of the spindling-sprout graft on sprout development of normal tubers is shown in figure 1, B.

The numbers of sprouts per hill from spindling-sprout tubers and from like tubers, core-grafted with buds from normal tubers, were also obtained.

Sprouts that developed from buds grafted into spindling-sprout tubers were normal as to size of stem and leaf development. These normal sprouts are not included among the data presented in table 1.

TABLE 1.—*Number of sprouts developed from, and yield of grafted and nongrafted tubers. Sprouts were counted at 40 days after planting. Yields were taken at 90 days after planting*

Planting material	Sprouts per plant	Yield per 50 plants
	No.	*Lb.*
Normal tubers	2.36 ± 0.24	33.7 ± 3.8
Normal tubers grafted with spindling-sprout cores (Graft A)	4.60 ± 0.24	41.2 ± 3.8
Spindling-sprout tubers grafted with normal cores (Graft B)	6.15 ± 0.38	12.8 ± 4.4
Spindling-sprout tubers	13.78 ± 0.35	8.9 ± 5.4

An interesting feature of table 1 is the smaller number of sprouts produced by spindling-sprout tubers grafted with a core of normal material than from nongrafted spindling-sprout tubers. Conversely, twice as many sprouts per plant were present on normal tubers grafted with spindling-sprout as on normal nongrafted tubers. That this increased number of sprouts is not a wound response has been demonstrated in greenhouse studies, whereby plugs were removed from normal tubers and plugs from normal tubers have been grafted into other normal tubers. Planted side by side in pots, the number of sprouts produced by the wounded or the grafted tubers equalled the number of sprouts formed by uninjured normal tubers.

Yields of the 4 classes of tubers are given in table 1. Because of the excessive amount of time that would have been required to harvest all replications, only 2 replications of the spindling-sprout tubers, and only 3 replications of the spindling-sprout tubers grafted with normal cores, were harvested. These facts accounts for the differences in the S.E.$_M$ of the yields. Significance of all data was determined by the method of analysis of variance of Snedecor (6).

Normal tubers, core-grafted with spindling-sprout plugs, outyielded normal nongrafted tubers, but the difference is not statistically significant. Likewise, there is no significant difference between the yields of spindling-sprout tubers and similar tubers grafted with cores from normal tubers. There is a significant reduction in yield due to spindling-sprout; tubers of this type yield less than 10 lb. per 50 plants.

Tubers produced from normal tubers were normal in all ways. Of the hills developed from normal tubers grafted with spindling-sprout cores (Graft A), 93.4 per cent produced normal tubers, 4.6 per cent produced tubers of normal size but prematurely sprouted, and 2 per cent produced tubers smaller than normal and prematurely sprouted. Sprouts were undersized, but could not be described as "spindly."

Many small tubers, less than ½ in. diameter, were produced by spindling-sprout tubers. Approximately half of these diminutive tubers were prematurely sprouted; but, though undersized, the sprouts were not thread-like, while the other 50 per cent of the tubers were dormant. Progeny, produced from the normal buds, introduced into spindling-sprout tubers (Graft B), were of normal size and not sprouted, whereas the progeny of the spindling-sprout tubers themselves were similar to the progeny of non-grafted spindling-sprout tubers described above.

The undersize progeny of spindling-sprout tubers were segregated into sprouted and nonsprouted; the latter were treated with ethylene chlorohydrine to break their dormancy, and approximately 25 tubers of each class were planted side by side in the field. All plants that developed were distinctly undersize, but the nonsprouted tubers produced more nearly normal plants than tubers, which were sprouting when harvested. Fifty days after planting, the number of sprouts per hill were counted, and it was found that tubers, sprouted when harvested, produced almost twice as many stems as those that were not sprouted when harvested. Sprouts from tubers of the former type were profusely branched, as on potatoes diseased with witches' broom. Sprouts from other plants were normal as to branching.

The hills were harvested at 90 days and the tubers were counted and measured. None of the tubers formed from nonsprouted seed was sprouting, whereas almost all of those from sprouting seed were prematurely sprouted. The sprouts again could not be called "spindly." All data are given in table 2.

TABLE 2.—*Results of planting the sprouted and unsprouted progeny produced by spindling-sprout tubers. Sprouts were counted at 50 days after planting. Yields were taken at 90 days after planting*

Type of progeny	Number of tubers planted	Number of sprouts per hill	Number of branches per sprout	Number of tubers per hill	
				Greater than ½″ diameter	Smaller than ½″ diameter
Not sprouted when harvested	25	1.5	1.4	7.0	2.8
Sprouted when harvested	25	2.4	3.1	2.0	12.4

To determine further whether the dormant progeny of spindling-sprout tubers possessed the substance or substances responsible for the subsequent development of spindling-sprout, normal-size, normally germinating, Bliss Triumph tubers were grafted with cores taken from the dormant seed mentioned above, and planted in pots in the greenhouse. As checks, normal tubers, grafted with cores from normal tubers, and normal tubers with a plug of tissue removed, were planted at the same time. It was found that the growth, type, and number of stems per plant, of the three types of tubers, were identical. No data were taken on yields.

This result checks with previous observations and seems to indicate that it is possible to eliminate, by selection at harvesting, spindling-sprout from a potato-tuber population, affected with this disease. Leach (3) is in agreement on this point when he states "when tubers affected with spindling-sprout are removed, the value of the remaining tubers seems to be unimpaired."

Chemical Analyses

In table 3 are presented the chemical compositions of the original spindling-sprout tubers and normal tubers as received in Hawaii. There was a greater concentration of reducing sugar, sucrose, acid-hydrolyzable materials, soluble nitrogen, ammonia, and amino nitrogen in spindling-sprout tubers than in normal tubers. For the first two of these fractions the differences seem large enough to be considered possibly significant, but, for the other differences, significance is questionable.

TABLE 3.—*Chemical compositions of spindling-sprout tubers and normal tubers from the same bag, variety Bliss Triumph. (All data, except water, given as percentage of dry weight)*

Fraction	Spindling-sprout tubers	Normal tubers
	Per cent	*Per cent*
Water	79.02	75.38
Soluble solids	17.10	12.12
Glucose	4.51	2.03
Sucrose	4.48	1.93
Total sugar	8.99	3.96
Acid-hydrolyzable	73.80	71.80
Soluble nitrogen	0.74	0.65
Insoluble nitrogen	0.74	0.98
Total nitrogen	1.48	1.63
Ammonia nitrogen	0.0246 (3.32)[a]	0.0158 (2.43)[a]
Amide nitrogen	0.0773 (10.45)	0.0718 (11.04)
Amino nitrogen	0.4528 (59.98)	0.3542 (54.26)

[a] Data in parentheses are soluble-nitrogen fractions expressed as percentage of total soluble nitrogen. The undetermined fractions were 26.25 per cent and 32.27 per cent for spindling-sprout and normal tubers, respectively.

Inability of the apical meristems of the buds of the tuber to synthesize proteins may be the cause of the accumulation of soluble carbohydrates and soluble nitrogen (ammonia and amino nitrogen) and the decrease in insoluble material in the spindling-sprout tubers. Although this might cause the formation of vitiated sprouts, the underlying reason or reasons for the abnormal physiology are not known.

SUMMARY

Investigations were conducted with Bliss Triumph potatoes affected with spindling sprout to determine the nature of this disease. In the present studies, spindling sprout is defined as the premature production of thread-like sprouts by tubers. When affected tubers are planted, yields are much less than when normal tubers are planted.

Spindling sprout is not transmitted by grafting. When normal tubers are grafted with cores taken from spindling-sprout tubers, the eyes of the normal tuber are stimulated and a larger number of healthy stems are produced than are formed by normal tubers, or by normal tubers grafted with cores from normal tubers. Eyes from normal tubers grafted into spindling-sprout tubers form shoots that are normal in leaf and stem development.

Yields of normal tubers grafted with spindling-sprout cores were higher than yields of normal nongrafted tubers or normal tubers grafted with normal cores. The differences found are not statistically significant.

The progeny of spindling-sprout tubers may be prematurely sprouted or dormant at harvest; the sprouts are not threadlike or spindly, though they are smaller than normal. When planted, nonsprouted progeny behave like normal plants, whereas sprouted progeny produce sprouted tubers. These sprouts are again smaller than normal, but are not threadlike or spindly.

Indications were found that it is possible to eliminate spindling sprout from a tuber population by selection at harvesting.

A chemical analysis of spindling-sprout tubers and of normal tubers from the same bag of seed is presented. Spindling-sprout tubers contained a greater concentration of reducing sugar, sucrose, acid-hydrolyzable materials, soluble nitrogen, ammonia, and amino nitrogen than was found in normal tubers. For the first two fractions mentioned, the differences found are large enough to be considered possibly significant.

Spindling sprout may possibly be due to an inability of the apical meristems of the buds of a tuber to synthesize proteins. The reason or reasons for the abnormal physiology still remain to be demonstrated.

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LITERATURE CITED

1. GILBERT, A. H. Correlation of foliage degeneration diseases of the Irish potato with variations of the tuber and sprout. Jour. Agr. Res. [U. S.] 25: 255–266. 1923.
2. JONES, W. W. Respiration and metabolism in etiolated wheat seedlings as influenced by phosphorus nutrition. Plant Physiol. 11: 565–582. 1936.
3. LEACH, J. G. The biological basis for certification of seed potatoes. Amer. Potato Jour. 15: 117–130. 1938.
4. MORRIS, H. E., and P. A. YOUNG. Potato diseases in Montana. Montana Agr. Exp. Stat. Bull. 300. 1935.
5. PREGL, F. Quantitative organic microanalysis. 237 pp. P. Blakiston's Son and Co. (Philadelphia). 1930.
6. SNEDECOR, G. W. Calculation and interpretation of analysis of variance and covariance. 96 pp. Collegiate Press, Inc. (Ames, Iowa). 1934.
7. STEWART, F. C., and F. A. SIRRINE. The spindling-sprout disease of potato. New York (Geneva) Agr. Exp. Stat. Bull. 399: 133–143. 1915.
8. STILES, H. R., W. H. PETERSON, and E. B. FRED. A rapid method for the determination of sugar in bacterial cultures. Jour. Bact. 12: 427–439. 1926.
9. STUART, N. W. Nitrogen and carbohydrate metabolism of young apple trees as affected by excessive applications of sodium nitrate. New Hampshire Agr. Exp. Stat. Tech. Bull. 50. 1932.
10. WERNER, H. O. Relative productivity of seed potatoes grown under various controlled environmental conditions. Jour. Agr. Res. [U. S.] 38: 405–410. 1929.

THE BLACK ROT OF BARBAREA VULGARIS

Walter H. Burkholder

(Accepted for publication September 11, 1940)

During the last 10 years the black rot of cabbage and cauliflower, caused by *Phytomonas campestris* (Pammel) Bergey *et al.* has not been a serious problem in New York State. This has been so because most of the seed planted has undergone the hot-water treatment, which destroys the bacterial pathogen. The severe systemic symptoms of the black rot have disappeared from the State except in isolated cases. One symptom of the disease, however, is still present, especially on cauliflower. This is a late infection of the leaves, manifest as numerous local lesions. The appearance of this type of black rot is similar to the peppery spot of cauliflower caused by *Phytomonas maculicola* (McCulloch) Bergey *et al.*, and has been described from Long Island by Clayton.[1] Many isolations have been made by the writer from these small local lesions, which appear late in the season, and in all cases *Phyt. campestris* has been obtained. *Phyt. maculicola* has been isolated several times and only then from specimens collected early in the season. Twenty-three isolates of *Phyt. campestris* from these local lesions have been shown able to produce typical systemic black rot in cauliflower and cabbage. There is no evidence of strain differences.

Since black rot is not present in the cauliflower fields up to August and, after that, only as local lesions, the question arises: whence comes the primary inoculum? Not from the cauliflower seed or seedlings, because, were such the case, the disease would appear in typical black-rot symptoms early in the growing period. One answer to the question might be that the inoculum is carried from diseased crucifer weed hosts near the cauliflower fields. The pathogen is known to infect *Brassica arvensis,* which is an annual and would not be able to over-winter the organism. It likewise infects *B. nigra,* another annual, which also at times may be a winter annual. Under the latter condition, however, this plant dies early in the season after producing seed. These hosts, therefore, could hardly harbor the pathogen, which gives rise to late infection. Furthermore, the writer has never been able to obtain more than light infection on these 2 species of *Brassica* with *Phytomonas campestris.* One crucifer weed, ubiquitous in New York State and a biennial, or perennial, is winter cress, *Barbarea vulgaris* R. Br. Consequently, this plant fell under suspicion, and a search for a disease on it, other than the universal white spot caused by *Ramularia barbareae* Pk., was undertaken. Such a disease was found readily, which produced in appearance black rot symptoms on the leaves. Small spots, at first green and water-soaked and later blackish, were observed. These lesions extended down the stem and killed the leaves. Examinations of the lesions showed them to be full of bacteria, and isolations revealed that the bacteria in culture were identical in appearance with *Phyt. campestris.* Two isolations from cauliflower and 4 from winter cress gave the following characteristics.

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[1] Clayton, E. E. Studies of the black-rot or blight disease of cauliflower. New York State Agr. Exp. Stat. Bull. 576. 1929.

Rods 1.0 to 3.15 by 0.4 to 0.95 μ. Motile, with a single polar flagellum. Gram-negative. On beef-extract peptone agar, colonies circular, yellow, smooth, butyrous, growth moderate. Growth on potato-dextrose agar abundant, pale yellow and mucoid. Broth becomes turbid, with yellow granular ring. Gelatin liquefied. In milk, litmus is reduced, there is light curd formation with clearing and production of tyrosine crystals. No indol. H_2S produced. Nitrate utilized but not reduced to nitrites. Asparagine not utilized. Acid from dextrose, galactose, xylose, glycerol, maltose and sucrose. Utilization of salts of malonic, malic, citric, and succinic acid with alkaline production. No utilization of rhamnose, salicin, nor hippuric acid. Starch hydrolyzed. Aerobic.

Inoculation experiments, however, proved the winter-cress organisms to be different from the cauliflower pathogen. The isolates from *Barbarea vulgaris* produced the typical disease on its host plant, but not on horseradish and but very slight to no infection on cauliflower. The cauliflower organism produced good infection on its host plant but no infection on *B. vulgaris* and very slight infection to none on the horseradish. These experiments were carried out under favorable conditions of moisture, and the plants were injured slightly by pricking with insect-mounting needles. Where a slight infection seemed to occur on cauliflower, after inoculation with the *Barbarea* organism, examinations showed no increase of bacteria in the lesions but rather a diminishing number.

During this investigation several isolates of *Phytomonas campestris* var. *armoraciae* (McCulloch) Bergey *et al.* were obtained from diseased horseradish. Their pathogenicity was proved on this host, and inoculations were made on cauliflower and winter cress. On these two latter plants infection was none or very slight. Thus the 3 pathogens were shown to be different pathologically.

R. Lincoln endeavored through repeated inoculations of cauliflower with the *Barbarea* organism to build up a strain that would produce a disease in this latter plant. He was unsuccessful, however. The possibility of such a change occurring in the field during the growing season seems, therefore, rather slight.

Since the pathogen from *Barbarea vulgaris* is distinct from other organisms as far as one can ascertain, it seems desirable to give it a name. **Phytomonas barbareae**, n. sp. is proposed. If one accepts Dowson's new genus, *Xanthomonas*[2] and this should be considered seriously, the name would be *Xanthomonas barbareae.*

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[2] Dowson, W. J. On the systematic position and generic names of the Gram-negative bacterial plant pathogens. Zentralb. f. Bakt. Par. und Infek. II Ab. 100: 177–193. 1939.

PHYTOPATHOLOGICAL NOTES

Rosy Canker of London Plane Associated with Illuminating-gas Injury.—In connection with studies of canker and wood stain (*Ceratostomella* sp.) of the London plane, another striking canker has come under the writers' observations. The condition was first observed in Baltimore, Maryland, in 1939. It has since been seen on Staten Island, New York, and in New Jersey. Some of these locations were called to our attention by P. P. Pirone of the New Jersey Agricultural Experiment Station, E. G. Rex of the New Jersey State Department of Agriculture, and E. A. Rundlett of the New York City Department of Parks.

In 7 of 8 locations it has been definitely established that illuminating gas had escaped into the soil near the affected trees. For the other location there is no unquestionable evidence yet available concerning gas leakage. The writers believe that this canker probably is a response to poisoning by illuminating gas in the soil.

The cankers are very striking with their furrowed, hypertrophied, more or less decorticated centers composed of rosy to brown proliferated tissue (Fig. 1). The bark is pushed away from the wood by an excessive proliferation, which forms a soft, watery, rose-color mass of tissue composed of thin-walled cells. The color changes from rose to brown upon exposure to the air. Because of the distinctive color, the name rosy canker is suggested. The centers of cankers from which the bark has fallen do not long remain rose-color, but, at the margin of the canker, where the bark has been raised, though not loosened, the rose color persists in the proliferated tissue. If the soft proliferated tissue, which in some cases may be $\frac{3}{8}$ in. thick, reminding one of the flesh of a watermelon, has not been too long exposed to drying, it can be scraped away easily with the finger. Sometimes there is a slight odor of fermentation associated with the soft tissue. After drying, the proliferated tissue becomes hard, brown, and roughly furrowed. This dry, brown tissue can be recognized on some trees at least a year after the cankers have formed.

The branch or trunk cankers are of irregular shape, but tend to be elongate. Early stages of the cankers are evidenced by slight swellings with longitudinal and transverse fracturing of the bark. Sometimes a faint to distinct pink to rose color can be found in the bark where no external evidence of canker can be seen.

Stone[1] described a somewhat similar canker on *Populus deltoides* Marsh. that was called to his attention by James Walker of Newark, N. J. Stone stated, "the tissue was perfectly white and fresh in appearance, was easily broken and apparently as delicate as the tissue of an apple. Later the tissue turned brown and disintegrated and became slimy in appearance." Stone also remarked that on willow cuttings growing in water charged with gas, the bark often split and a mucilaginous mass formed under the bark.

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[1] Stone, G. E. Effects of illuminating gas on vegetation. Ann. Rept. Mass. Agr. Exp. Sta. 25: 45–60. 1913.

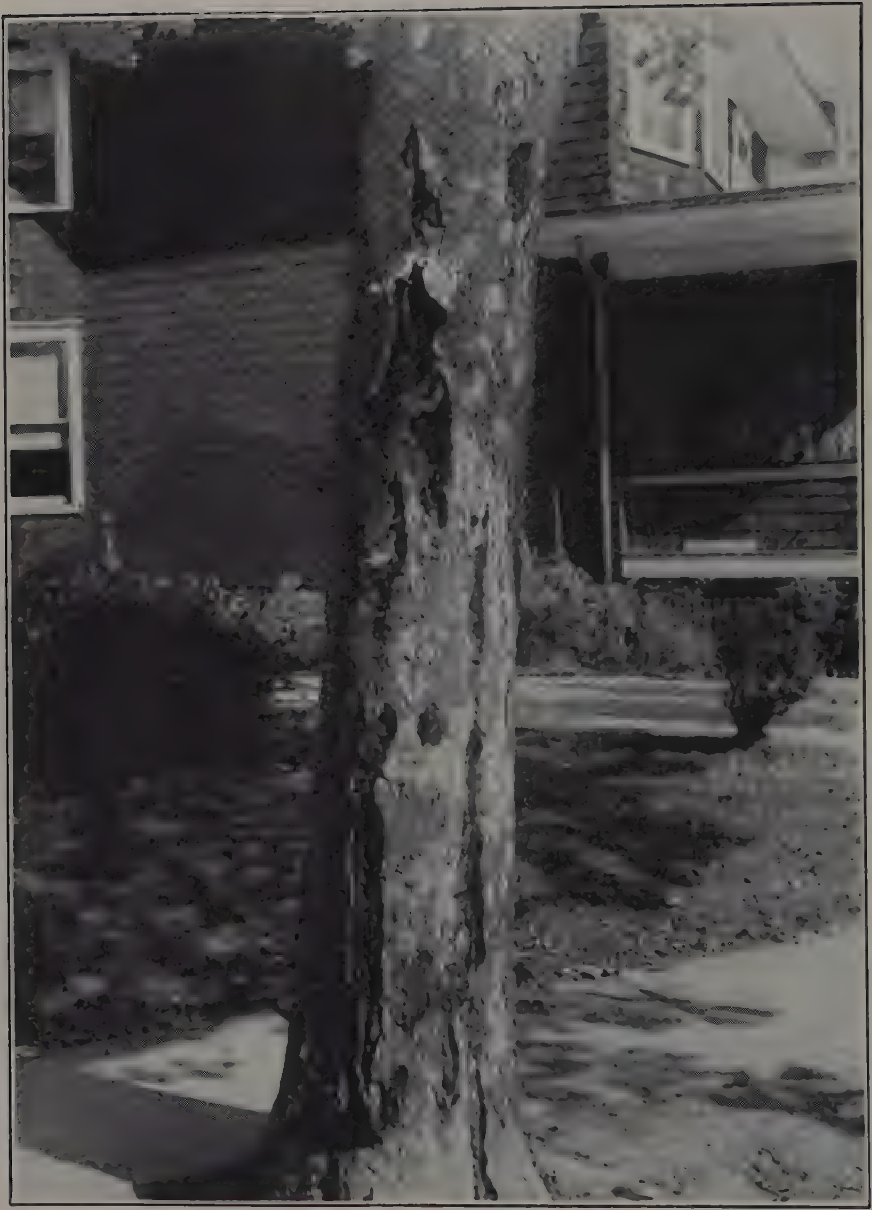

FIG. 1. Trunk of London planetree with typical rosy canker. The bark had cracked from the wood and curled back. The centers of the cankers were covered with the dry fissured remains of the formerly soft rosy proliferated tissue. Rosy tissue was present at the canker margins. Photograph by P. V. Mook.

The top of the stump of one severely cankered London plane showed brownish-pink spots in the wood of a few of the outer annual increments and scattered, diffused, pink spots nearer the center. On making smooth cuts across the central portion of the stump, rich-red, pin-point spots surrounded by nondiscolored wood were disclosed. Droplets of red liquid exuded at these points and enlarged to pinhead size before spreading over the smoothed transverse section to form pink blotches. On this tree some branches 1 in. in diameter had a pale-pink discoloration of the inner bark and of the cambial region 20 ft. from the nearest recognized canker.

Numerous platings of the inner portions of the proliferated tissue and the pink wood have been made, but the material has regularly proved sterile.

Six trees, found to have severe cankering of the above-described type, are known to have died during the past year. Sixteen cases, with slight to moderate cankering, are still under observation. On one street several Norway maples have been killed by the escaping gas but the London planes, severely cankered, are still alive. Further study may show that planetrees with early stages of this type of canker can be saved by promptly stopping the gas leak and aerating the soil.—C. MAY, J. M. WALTER, and P. V. MOOK, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, N. J.

Resistance to Floral-infecting Loose Smut (Ustilago nuda) in Fall-sown Barley Varieties at Statesville, North Carolina.—Nursery records at the Piedmont Branch Experiment Station, Statesville, N. C., during the past 8 years have shown a prevalence of loose smut of barley in that area and a difference in varietal susceptibility to the disease.

To obtain more specific information on certain varieties, floral-inoculation studies were carried on in the 3-year period 1937–1939. Ten strains of barley were used, though not all were inoculated each year. Locally collected inoculum, identified as the floral-infecting loose smut (*Ustilago nuda*) by V. F. Tapke of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, was used throughout the experiment. Moore's[1] partial-vacuum method was used in making the inoculations.

Heads of approximately the same degree of development were selected and tagged as they were inoculated. When these were mature they were harvested and threshed by hand. Seed from each head was sown in a separate 5-foot row that fall and the percentage of infected heads determined the following spring. A summary of the results secured for the entire period is given in table 1.

The data show a decided difference in varietal susceptibility, but would have been more conclusive had there been less variation in the results within the individual varieties. Even in the most susceptible strains less than 50 per cent of the inoculated heads produced smutted plants the following year, and, where smut did appear, the percentage of plants affected varied from 72 to a trace.

This method of inoculation was found to be rather rapid (two people working together could easily inoculate 30 heads per hour), and would be

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[1] Moore, M. B. A partial vacuum method for the inoculation of wheat and barley with loose smut. Phytopath. **26**: 103. 1936.

TABLE 1.—*Summary of results of inoculating 10 varieties of barley with Ustilago nuda*

Variety or strain	Total number of heads inoculated	Percentage inoculated heads producing smutted plants
N. C. Bearded 70	20	45.0
Tennessee Hooded	74	36.5
N. C. Bearded 68	78	34.6
N. C. Bearded 83	10	20.0
Tenn. Winter	10	10.0
N. C. Beardless 26	75	6.7
N. C. Beardless 23	6	0.0
N. C. Bearded 11	6	0.0
N. C. Bearded 15	17	0.0
Odessa	3	0.0

very useful if more consistent results could be obtained. Thinking that some improvement might be made by using heads of the same degree of maturity, 3 of these barleys were chosen and 70 heads of each selected and tagged just as their heads broke through the boot. Ten heads of each variety were then inoculated on each of 7 successive days. During this period there was neither rain nor abnormal changes in temperature.

The best results were secured from the second to the fifth day with the peak occurring on the fourth. From inoculations on this day, 6 out of 9 heads of Tennessee Hooded and 7 out of 10 of N. C. Bearded 68 produced some smutted plants the following season. In those head rows where smut did occur, however, the percentage of heads infected varied from 3.33 to 72.41 and from 5.07 to 61.54 for these two strains, respectively, with the average percentage being only 17.1 and 14.8. The third strain, N. C. Beardless 26, produced no smutted plants in this experiment.

While the selection of heads of uniform maturity seemed to increase the effectiveness of the inoculation to some extent, there is need of still further improvement in technique under the conditions of this work. This will be especially important when working with varieties that are only slightly susceptible or that have been found difficult to infect. An illustration of this was observed in 1940 while carrying on further studies with the 4 strains listed above that failed to produce any smutted plants in previous years. In the spring of 1939 these were inoculated by Tapke's[2] method of puncturing the glumes and introducing the inoculum with forceps. By this method some infection was obtained with 2 of these 4 strains; 7.9 per cent of all heads of Odessa and 1.2 per cent of N. C. Bearded 15 smutting in 1940.

This is not a comparison of the efficiency of the two methods, as they were used in different years and a larger number of heads were inoculated by the latter method. It does, however, further emphasize the need of improved technique if fine distinctions are to be made between varieties.—

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[2] Tapke, V. F. A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Res. [U. S.] 31: 491–568. 1935.

G. K. MIDDLETON and W. H. CHAPMAN, Agronomy Department, N. C. Agricultural Experiment Station.

A New Case of Rosette Mosaic on Peach.—In 1938, a peach tree was found in Rockland County, New York, with symptoms hitherto not observed in that State. The stunted character of the affected tree was in striking contrast to that of the normal, surrounding trees. Extreme shortening of the internodes, with consequent crowding of the foliage, resulted in a rosette type of growth. Although resembling in some respects peach rosette, an old and widely known virus malady, it differed in being much less severe on the tree and in leaf characters. Instead of chlorosis

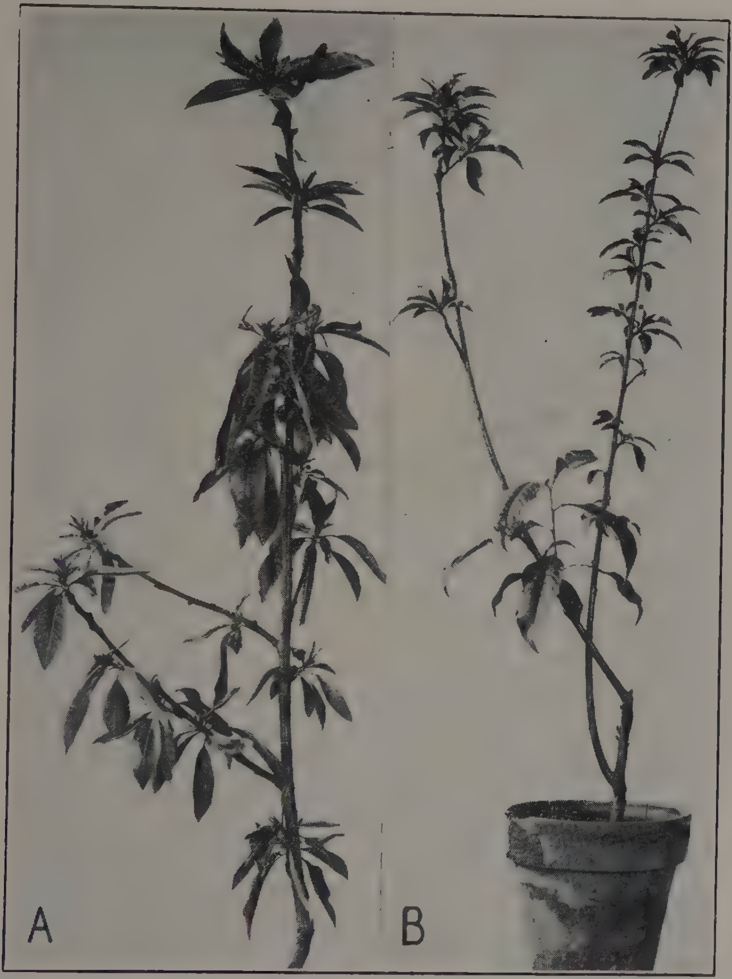

FIG. 1. A. Rosette on Elberta peach about 7 months after budding, showing typical symptoms of rosetting and stunting of the terminals and the upward rolling of the leaves. B. Rosette mosaic on Elberta peach 7 months after budding, showing rosetting of a different character than in A, stunting of the terminals and the typical wavy-leaf condition. At this stage the leaves have recovered from the mosaic symptom shown in figure 2.

and curling or inward rolling of the leaves, as in peach rosette (Fig. 1, A), there was a peculiar wavy type of growth, apparently identical with what has been called by Cation[1] Michigan rosette or rosette mosaic.

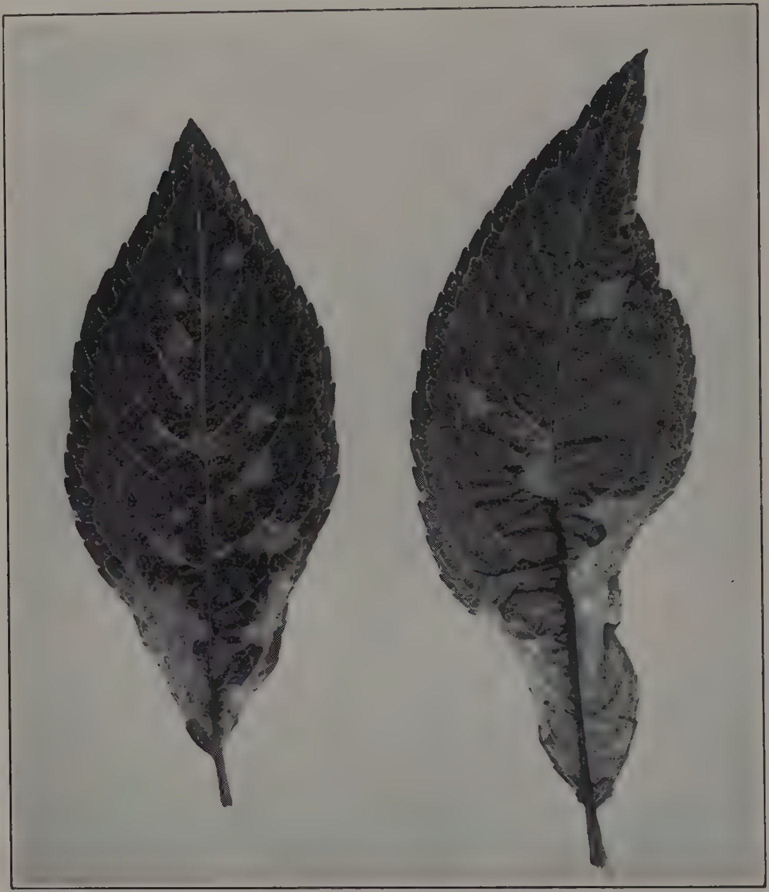

FIG. 2. In rosette mosaic, distortion and local chlorosis or mosaic are of frequent occurrence, especially on the younger leaves, which develop at the cooler temperatures of early spring.

Transmission to healthy Elberta peach trees in the greenhouse was readily accomplished by budding. All 10 trees, inoculated during late summer, showed symptoms shortly after starting growth the following season. The inoculated trees, when stored outdoors until January 15, and then brought inside, were showing symptoms on February 17. The first symptom of leaf distortion was accompanied within a week by chlorotic patterns or mosaic of the leaf blade (Fig. 2). Both chlorotic spots and leaf distortion were present on some but not all the leaves when the temperature averaged below 70° F. Similar symptoms were obtained with bud

[1] Cation, Donald. An infectious rosette of peach trees. Mich. State Quar. Bull. **16**: 79–84. 1933.

wood of rosette mosaic from Michigan, leaving no doubt as to the identity of the trouble.

The mosaic symptom gradually disappeared as the season advanced, whereas the wavy character of many of the leaves, rosetting of the foliage, and stunting of the trees persisted. A typical affected tree on March 25 is illustrated in figure 1, B. Certain of the inoculated trees produced fruit that ripened during the first week of May. The ripe fruit was of uniform naphthalene-yellow[2] and of good taste. On the same date the fruit on several adjacent healthy trees was still immature.

The grower concerned had noted this peculiar tree for several seasons prior to 1938. In view of the fact that no spread has occurred it is suggested that the vector responsible for its dissemination in Michigan may not be present in New York.

In prune mosaic[3] a somewhat similar wavy-leaf condition, occasionally accompanied by chlorotic spots, has been induced on Elberta peach by budding from the prune (Hildebrand, unpublished). The effect of prune mosaic on the peach was much milder than that induced by rosette mosaic, and the peach trees appeared largely to recover from the former but not from the latter. While important differences exist, it is possible that the viruses involved may be related.—E. M. HILDEBRAND, Cornell University, Ithaca, N. Y.

Cherry Yellows (Physiological Yellow Leaf) in New York.—Yellow leaf of the sour or pie cherry (*Prunus cerasus* L.) was first described as a nonparasitic disease by Stewart.[1] Occasionally the leaves were observed to turn yellow and fall prematurely in large numbers. It was distinguished from leaf spot (*Coccomyces hiemalis* Higgins) by the absence of leaf lesions and was believed to be caused by unfavorable soil and weather conditions, since it was not preventable by spraying. Gloyer and Glasgow[2] found the so-called physiological yellow leaf affecting as high as 60 per cent of the foliage of cherry trees, and observed that it occurred independently of the leaf spot and was not influenced by applications of fungicides. Furthermore, the points were stressed that it was particularly associated with nonvigorous trees growing in poorly drained locations and often with trees suffering from winter injury. They noted also that some of the leaves became yellow only in part.

Plant-disease-survey records at Cornell reveal the presence of physiological yellow leaf in New York every year since 1928. In that year it caused a heavy leaf drop in individual trees and similar heavy defoliation was recorded for 1931, 1935, 1937, and 1938, with less apparent damage in the intervening years.

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[2] Ridgway, R. Color standards and color nomenclature (Washington). 44 p., 53 colored plates. 1912.

[3] Thomas, H. Earl, and E. M. Hildebrand. A virus disease of prune. Phytopath. 26: 1145–1148. 1936.

[1] Stewart, F. C. Notes on New York plant diseases, II. New York (Geneva) Agr. Exp. Stat. Bull. 463. 1919.

[2] Gloyer, W. O., and H. Glasgow. Defoliation of cherry trees in relation to winter injury. New York (Geneva) Agr. Exp. Stat. Bull. 555: 27 p. 1928.

Its earliest recorded appearance during the respective seasons varied from June 16 to July 6. By July 15 leaf fall had usually terminated for the season. Marked trees in Wayne County have shown the trouble each severe year since 1930.

This so-called physiological yellow leaf has been observed in other cherry-producing States including Michigan and Wisconsin. The time of leaf fall and the character of the injury agree closely between the States. What had previously been called physiological yellow leaf or "boarder tree" in Wisconsin was recently demonstrated to be transmissible by budding and therefore of virus origin (Keitt and Clayton[3,4]). Because of unforeseen circumstances, the results of the original transmission tests started here in 1935 were lost. However, based on transmission tests now in progress and on observations made by the writers over a period of years in New York and on two occasions in Wisconsin during the 1940 season by the senior writer, little doubt remains that the same disease is involved in both places and that physiological yellow leaf is the cherry-yellows virus disease. Cherry nursery stock imported into Wisconsin from another State in the spring of 1940 was showing yellow-leaf symptoms only a few months after planting on the occasion of the aforementioned visit. The appearance of symptoms so soon after planting is good evidence that the young trees contained the virus when planted. Apparently the nurserymen are not aware of the disease in nursery stock which may account for its widespread distribution.

Although agreeing rather closely in leaf symptoms and time of leaf fall, affected trees in New York seem to suffer less than in Wisconsin from the more severe type of injury, which greatly reduces the spur system and fruit yields. As a result the light-bearing "boarder" type of tree characterized by its reduced spur system, lighter green foliage and stubby erect twig growth has been less in evidence in this State. While many of the facts are not known it is possible that the more severe climate of Wisconsin may intensify the effects of the disease.

Studies now in progress may possibly clarify these points.—E. M. HILDEBRAND and W. D. MILLS, Dept. of Plant Pathology, Cornell University, Ithaca, New York.

Citrus Viruses.—A simple, easily applied pro tem. manner of naming viruses[1] has been suggested recently with proposals for names for certain

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[3] Keitt, G. W., and C. N. Clayton. A destructive bud-transmissible disease of sour cherry in Wisconsin. Phytopath. 29: 821–822. 1939.

[4] ——— and ———. A bud-transmissible chlorosis of *Prunus cerasus*. Phytopath. (Abstract) 30: 13. 1940.

[1] Fawcett, Howard S. Suggestions on virus nomenclature as exemplified by names for citrus viruses. Science 92: 559–561. 1940.

citrus viruses. The rule for names of pro tem. genera is to "*add the stem 'vir' for virus* (Latin neuter) *to the Latin genitive of the genus of the host in which the virus was first discovered and recognized, dropping any final consonants that occur in this genitive.*" This gives to citrus viruses the genus, *Citrivir,* meaning a virus of citrus. The description of citrus viruses, the transmission of which has been determined, are:

Citrivir italicum (italicum = pertaining to Italy). The virus causing infectious mottling of citrus. Causes white, pale-green, or yellow irregular areas in leaves, leaving narrow green bands along midrib, as described by Petri.[2] Leaves also blistered and misshapen. Found only on sour orange leaves associated with *Toxoptera aurantidi* in Italy by Petri, who considered this aphis to be the vector. No physical or chemical properties of the virus known.

Citrivir psorosis (psorosis, Latin genitive of psorosis = of the disease known as psorosis). The virus causing psorosis of citrus.[3, 4] Induces on young, tender leaves small elongated, light-color areas or flecks in the region of the small veinlets and sometimes warping of leaves. Sometimes causes veinlet clearing and irregular zones of clearing in an oak-leaf-like or other type of pattern. Also causes scaling of outer layers of bark or depressions and deformities in bark and wood. Causes spotting on mature leaves rarely except with var. *anulatum.* Has been transmitted only by tissue fusion. No vector known except man.

Var. *Vulgare* (vulgare = general, common). The virus of psorosis A, or the common scaly bark type.[3, 4] In addition to the young leaf symptoms, common to all varieties, this variety causes slow scaling or pustular eruptions of outer layers of bark in limited areas, with or without exudation of gum. Later, produces a drab-gray, cinnamon-drab to rufus discoloration of the wood, accompanied by the decline of the tree. Transmitted by tissue fusion. World-wide in distribution.

Var. *anulatum* (anulatum = with a ring). The virus of psorosis B, a distinctly different type from A (Fawcett[3, 4]). Causes a rapid scaling of outer bark in continuous areas that progresses very rapidly along one side of trunk or branch causing necrosis. Gum exudation in advance of scaling. Large circular spots, frequently in form of rings or zones, sometimes concentric, on fruit and mature leaves. Causes also circular or semicircular furrows and bumps on some fruit. Trees decline rapidly after initial scaling. Occurs only occasionally in California. Leaf and fruit symptoms attributable to this virus are similar to zonate chlorosis of Brazil. Transmitted by tissue fusion.

Other possible varieties or strains of the psorosis virus are those causing concave gum disease and blind pocket. The relationships of these are being further studied before adopting possible names such as var. *concavum* (concave) and var. *alveatum* (hollowed out like a trough).—HOWARD S. FAWCETT, Citrus Experiment Station, Riverside, California.

Powdery Mildew of Potato in Kentucky.[1]—During the fall of 1940 seedling potato plants that had been growing in 4-inch pots in the greenhouse at the Kentucky Agricultural Experiment Station since the previous spring were observed affected by a powdery mildew. The leaves were covered with powdery masses of spores on both sides. Only leaves on old shoots were affected, many new shoots were entirely free from visible infection. Before the leaves died perithecia developed on a few of them.

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[2] Petri, T. Variegatura infettiva della foglie "*Citrus vulgaris*" Risso. Bol. R. Staz. Pat. Veg. (n. s.) **11**: 105–114. 1931.

[3] Fawcett, Howard S. Psorosis: In citrus diseases and their control. (2nd Ed.) pp. 188–203. McGraw-Hill Book Co. (New York). 1936.

[4] ————. Is psorosis of citrus a virus disease? Phytopath. **24**: 659–668. 1934.

[1] Published by permission of the Director.

Conidia, 100 of which were measured, averaged 32.8 μ long by 13.8 μ wide, and varied from 25.5 to 38.2 μ long and 10.9 to 18.2 μ wide. This is slightly smaller than the spore size reported by Kunkel,[2] but this may be accounted for by the fact that the spores measured in the present instance were from leaves producing perithecia and were somewhat shrunken.

The perithecia were dark-brown, bore indeterminate hypha-like appendages, and measured 127 to 136 μ in diameter. Perithecia contained from 4 to 10 or more asci. Excepting a single trisporulate ascus, the asci were all bisporulate. Asci, 30 of which were measured, ranged in length from 54.5 to 72.7 μ and in width from 25.5 to 36.4 μ, with an average of 59.1 by 31.4 μ. The asci were more or less stalked. Ascospores averaged 21.3 by 13.8 μ and ranged in size in the 39 measured from 16.4 to 27.3 μ long by 10.9 to 20.0 μ wide.

The fungus was tentatively identified as *Erysiphe cichoracearum* DC. by the writer. Specimens sent to the Division of Mycology and Disease Survey were assigned by J. A. Stevenson to this species. He commented as follows: "The Kentucky specimen comes within the range of this species as delimited by Salmon. Those who would split up species complexes of this kind would certainly assign the Solanum species elsewhere. It is interesting to note that potato mildew in Europe has been referred to *Oidium solani* Auctores and *Erysiphe solani* Vanha, but no perithecia have ever been reported." H. A. Edson commented that "reports of *Erysiphe solani* on Irish potato have been received by the Survey from New Jersey and Oidium species from Pennsylvania. A specimen of mildew was received from the greenhouses at Beltsville in December, 1938."—W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

Inoculation of Cotton Plants in Sand Culture with Phymatotrichum Root Rot.—Under sand-culture conditions in the greenhouse, previous inoculations[1] of cotton plants with the root-rot fungus (*Phymatotrichum omnivorum* (Shear) Duggar) have not been wholly successful. Lesions were found on the lateral or tap roots, but the plants in sand often showed no aboveground symptoms of this infection. With soil-grown plants, however, inoculation with root rot has been successful[1, 2, 3, 4] under both field and greenhouse conditions.

In a recent experiment mature cotton plants, growing in washed sand under greenhouse conditions, have shown positive infection after inoculation with the cotton root-rot fungus. A successful inoculation technique of this sort might be useful in studying the effects of certain variables upon the susceptibility of cotton to root rot, under partly controlled conditions. With the technique used, more than 90 per cent of the plants showed wilting symptoms, indicating positive infection, within 3 weeks after inoculation.

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[2] Kunkel, L. O. Powdery mildew of potato in New Jersey. Phytopath. 26: 392–393. 1936.

[1] Blank, L. M. The susceptibility of cotton seedlings to *Phymatotrichum omnivorum*. Phytopath. 30: 1033–1041. 1940.

[2] Taubenhaus, J. J., and D. T. Killough. Texas root rot of cotton and methods of its control. Tex. Agr. Exp. Stat. Bull. 307. 1923.

[3] Taubenhaus, J. J., B. F. Dana, W. N. Ezekiel, W. J. Bach, and J. P. Lusk. A method of inoculation for Phymatotrichum root rot investigations. Phytopath. 19: 167–170. 1929.

[4] Neal, D. C., and G. T. Ratliffe. Infection experiments with the cotton root rot fungus, *Phymatotrichum omnivorum*. Jour. Agr. Res. [U. S.] 43: 681–691. 1931.

In this experiment, the cotton (var. Rogers' Acala) plants were grown in glazed jars filled with washed river sand with daily additions of 1 l. of a nutrient solution having the following approximate concentration: N—100 p.p.m.; P—30 p.p.m.; K—40 p.p.m.; Mg—10 p.p.m.; Ca—10 p.p.m.; B—1 p.p.m. These elements were supplied as NH_4NO_3, KH_2PO_4, $MgSO_4$ $Ca(NO_3)_2$, and H_3BO_3, respectively. The nutrient solution was adjusted to about 5.8 pH with sulphuric acid. Tap water (pH 8) was added to the plants as required between the applications of nutrient solution. Under greenhouse conditions, the soil temperature varied from 26 to 34° C. The size of the plants and the general appearance of those infected are shown in figure 1.

The inoculation procedure consisted in the placement of a small mass of

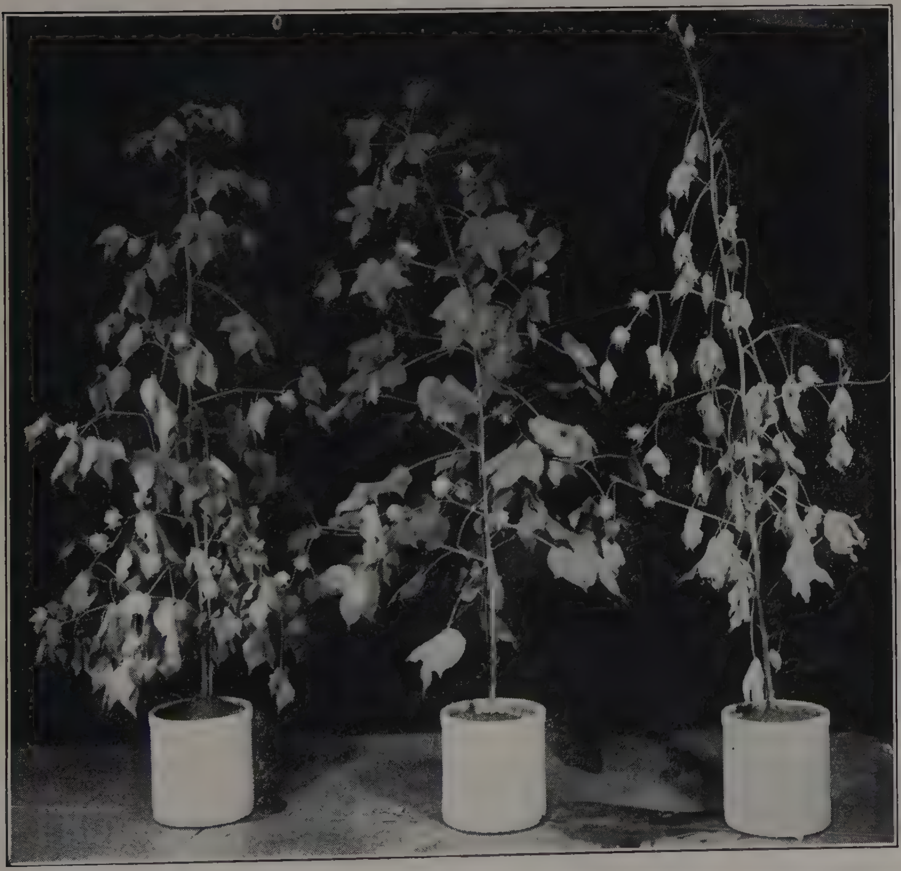

FIG. 1. Cotton plants in 3-gal. jars of sand showing results of Phymatotrichum root-rot infection, 2 weeks after inoculation. Left, plant showing first aboveground symptoms; center, control; right, advanced stage of infection. Note mound of soil at base of plants.

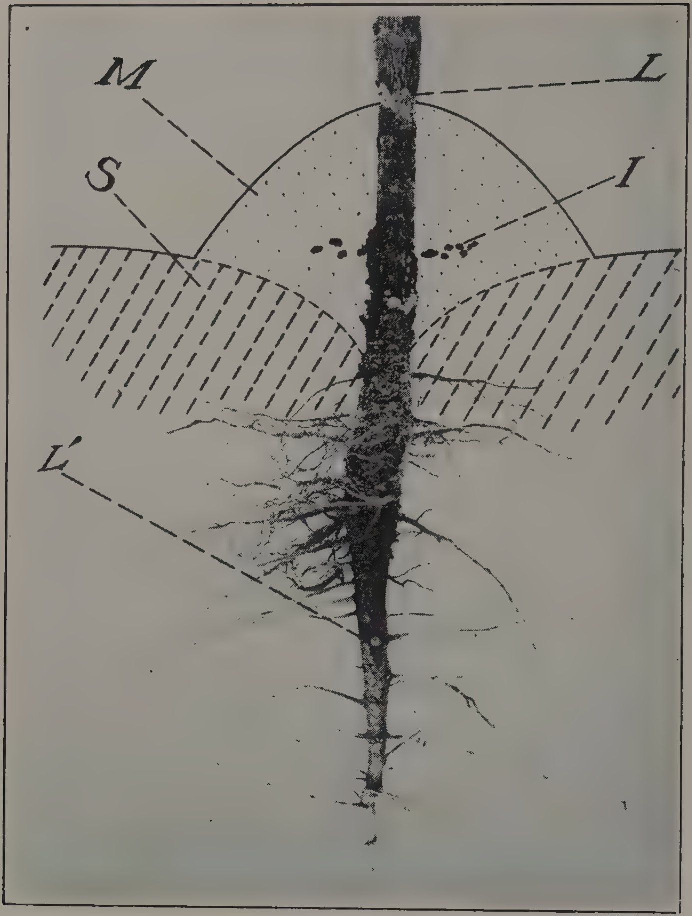

FIG. 2. Taproot and larger laterals of a sand-grown cotton plant showing infection with *P. omnivorum.* Note constriction, L, of stem at point of inoculation and the darkened lesion extending down the taproot. Other legend: S, washed sand (culture medium); M, mound of Houston soil; I, inoculum; L, lower extremity of taproot lesion.

soil and inoculum around the stem of the plant approximately at the surface of the sand culture. First, the surface sand was removed close to the cotton plant, exposing the stem down as far as the uppermost lateral roots. The depression in the sand thus formed around the stem was then filled with nonsterile Houston black clay, making a ring of soil around the stem about 2 cm. deep and 5 cm. wide. This highly calcareous soil medium favors especially the growth of the root-rot fungus. Several sclerotia[5] from 1-month-old sterile Houston soil (plus cotton seed or cowpeas) cultures were placed near the stem on this layer of soil. The pathogenicity of the isolate of *Phymatotrichum omnivorum* used had been demonstrated in other experiments.[6]

[5] Similar results with a few plants were obtained when pure cultures of the fungus on pieces of autoclaved cotton stem were used in place of the sclerotia as inoculum.

[6] See footnote 1.

Another layer of Houston soil was next placed directly on top of the previous layer and the inoculum, making a small mound from 6 to 8 cm. in depth, as is shown in figure 2. This mound of soil, with the inoculum imbedded near its center, was pressed firmly against the plant stem and also against the sand.

As a result of inoculating 75 3-month-old cotton plants by this method, 68 of the plants (91 per cent) were brought to a state of permanent wilting within 21 days after inoculation. The remaining 7 showed root lesions that probably would have caused the death of these plants eventually. On the average, the plants required 14.5 days to become permanently wilted. The first aboveground symptoms (wilting) appeared in some cases as early as 8 or 10 days after inoculation. A definite, constricted, cankered area covered with the brownish mycelium could be found completely surrounding the stem where the Houston soil came in contact with the plant, several days before wilting took place. In most cases, the infection as shown by the brown cankered area on the taproot had extended well down among the larger lateral roots by the time wilting had taken place (Fig. 2).—A. A. DUNLAP, Texas Agricultural Experiment Station, College Station, Texas.

Olive Knot Induced on Species of the Oleaceae by Artificial Inoculations. —*Phytomonas* (*Bacterium*) *savastanoi* (E.F.S.) Bergey, *et al.* is the causal organism of the olive knot on *Olea europaea.* Smith[1] has induced similar knots by *Phyt. savastanoi* on species of the Oleaceae: *Fraxinus velutinus, F. floribunda, Adelia* (*Forestiera*) *acuminata, Chionanthus virginica, Osmanthus aquifolium, Jasminum primulinum,* and *Olea chrysophylla.*[2]

Recently, d'Oliveira[3] reports knots produced artificially by *Phytomonas savastanoi* on *Fraxinus angustifolia, Forsythia intermedia, F. viridissima,* and on the wild olive of Portugal, *Olea europaea* var. *oleaster,* and *O. europaea* var. *sativa.* The inoculation of leaves of *Phillyrea media* produced rugose proliferations along the veins.

This paper reports some inoculations with *Phytomonas savastanoi* on the following species of the Oleaceae: *Forestiera neomexicana* Gray, *Ligustrum henryi* Hemsl., *L. japonica* Thunb., *L. lodense, L. nepalensis* Wall, *L. ovalifolium* Hassk., *L. sinensis* Lour., *Olea capensis* Linn., *O. ferruginea* Royle S.P.I. 22324, *O. laurifolia* Lam., *O. verrucosa* Link., S.P.I. 29047, *Osmanthus americanus* Benth. and Hook., *Os. aquifolium* Sieb., (*Os. ilicifolium* Hassk.), *Phillyrea decora* Boiss. and Bal., S.P.I. 1022541, *Syringa vulgaris* Linn., *S. persica* var. *laciniata* Vahl.

The inoculations were made with pure cultures of *Phytomonas savastanoi* on the current-year growth of plants growing in the open and in the lathhouse. Five inoculations in a series were made with controls. *Olea europaea* was included with the other species and was used to test the virulence of the organisms.

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[1] Smith, Clayton O. Pathogenicity of the olive-knot organism on hosts related to the olive. Phytopath. 12: 271–278. 1922.

[2] Smith, Clayton O. Olive knot on *Olea chrysophylla.* Phytopath. 24: 307–308. 1934.

[3] d'Oliveira, Maria de Lourdes. Inoculações experimentais com o *Bacterium savastanoi* E. F. Smith *e o Bacterium savastanoi* var. *fraxini* N. A. Brown. Agron. Lusit. 1: 88–102. 1939.

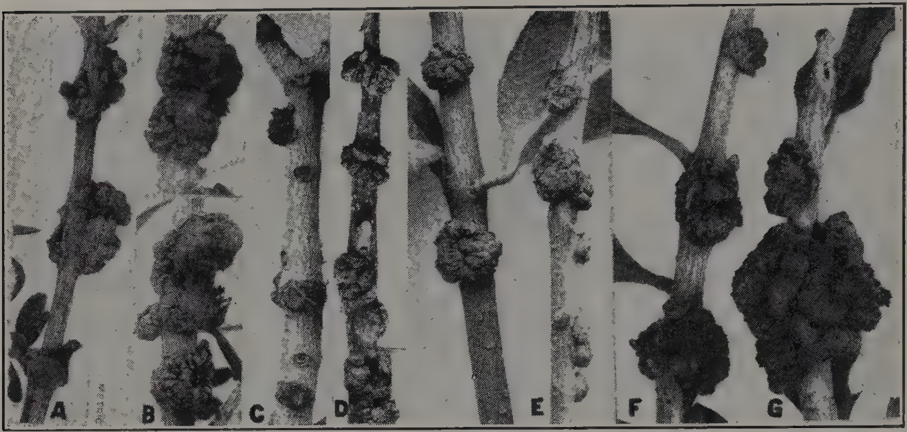

FIG. 1. Artificially induced knots on species of the Oleaceae. A. *Olea verrucosa* after 24 months. B. *O. ferruginea* (S.P.I. 22324) after 6 months. C. *O. laurifolia* after 6 months. D. *Forestiera neomexicana* after 3 months. E. *Osmanthus americanus* after 6 months. F. *Olea capensis* after 17 months. G. *Osmanthus aquifolium* after 17 months.

The results gave knots (Fig. 1) on *Olea capensis, O. verrucosa, O. ferruginea, O. laurifolia, Forestiera neomexicana, Osmanthus americanus,* and *Os. aquifolium.* Negative results were found on all the species tested of *Ligustrum* and *Syringa.* Typical knots developed on *Olea europaea. Phillyrea decora* developed abnormal overgrowths, which were suggestive, yet not typical knots. The controls healed in a normal manner.—CLAYTON O. SMITH, Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California.

REPORT OF THE THIRTY-SECOND ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE 1940 PHILADELPHIA MEETING

The thirty-second annual meeting of The American Phytopathological Society, held in Philadelphia, Pennsylvania (December 27 to 31, 1940), was one of the most outstandingly successful meetings the Society has held. Approximately 375 members were in attendance. Eighty-five new members were elected at Philadelphia, bringing the active membership roll to 1130.

More than 300 attended the Phytopathologists' Dinner at the Hotel Philadelphian, and enjoyed the program arranged by the committee under the able chairmanship of H. W. Thurston, Jr., of The Pennsylvania State College.

Special conferences were held on plant-disease survey, vegetable-seed-treatment research, recent studies on forage-crop diseases, and better undergraduate training for future plant pathologists. A very successful feature of the program was the invitation program of demonstrations, under the efficient direction of A. J. Riker, of the University of Wisconsin. The value of cooperation among different branches of plant science was brought out in joint sessions with Section G of the A. A. A. S. and affiliated botanical societies, American Association of Economic Entomologists, the Floriculture Section of The American Society for Horticultural Science, the Physiological Section of the Botanical

Society of America and The American Society of Plant Physiologists, the Potato Association of America, and the Mycological Society of America.

The summer session will be held in New Haven, Connecticut, June 23–27, 1941.

OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1941

Officers:

J. G. LEACH, President (1 yr.), West Virginia University, Morgantown, West Virginia.

L. M. HUTCHINS, Vice-President (1 yr.), P. O. Box 299, Brownwood, Texas.

R. S. KIRBY, Secretary (3 yrs. term expires 1943), The Pennsylvania State College, State College, Pennsylvania.

H. A. EDSON, Treasurer (3 yrs. term expires 1943), U. S. Department of Agriculture, Washington, D. C.

H. B. HUMPHREY, Editor in Chief of PHYTOPATHOLOGY (3 yrs. term expires 1943), U. S. Department of Agriculture, Washington, D. C.

Councilors:

J. B. KENDRICK, (term expires 1941), University of California, Davis, California.

CHARLES CHUPP (term expires 1941), Cornell University, Ithaca, New York.

J. G. HORSFALL (term expires 1942), Agricultural Experiment Station, New Haven, Connecticut.

N. J. GIDDINGS (Pacific Division), P. O. Box 752, Riverside, California.

GEORGE M. ARMSTRONG (Southern Division), Clemson Agricultural College, Clemson, South Carolina.

Representatives:

A. A. A. S. Council (1 yr.), C. R. ORTON, CHARLES CHUPP.

Elector Group V, Division of Biology and Agriculture, National Research Council, H. P. BARSS (E. C. STAKMAN, alternate).

Tropical Research Foundation (5 yrs. term expires 1945), ROBERT D. RANDS.

International Union of Biological Sciences, A. G. NEWHALL.

Board of Editors, American Journal of Botany (3 yrs. term expires 1943), G. W. KEITT.

Union of American Biological Societies (and Biological Abstracts), H. B. HUMPHREY and R. S. KIRBY (ex officio), F. V. RAND, C. W. BENNETT, H. A. EDSON, G. W. KEITT.

Standing Committees:

Advisory on Society Activities and Programs. W. J. ZAUMEYER, Chm., F. L. DRAYTON, A. A. DUNLAP, J. A. PINCKARD, R. K. VOORHEES, J. C. WALKER, C. E. YARWOOD.

Coordination in Seed Treatment Research. M. B. MOORE, Chm., C. H. ARNDT, H. T. COOK, F. J. GREANEY, C. M. HAENSELER, L. D. LEACH, G. L. MCNEW, P. P. PIRONE, H. A. RODENHISER.

Donations and Legacies. E. C. STAKMAN, Chm., J. G. BROWN, N. J. GIDDINGS, N. E. STEVENS, R. P. WHITE.

Extension Work and Relations. C. C. ALLISON, Chm., O. D. BURKE, R. J. HASKELL, G. W. KEITT, R. H. PORTER, OTTO REINKING, R. C. ROSE, D. R. SANDS, W. B. TISDALE.

Investments. H. A. EDSON, Chm., CHARLES BROOKS, MARVIN E. FOWLER, J. W. ROBERTS.

Necrology. A. G. JOHNSON, Chm., M. B. WAITE.

New Memberships and Subscriptions. R. F. POOLE, Chm., J. C. CARTER, KENNETH KADOW, R. S. KIRBY (ex officio), L. D. LEACH, R. M. LINDGREN.

Nomenclature and Classification of Plant Viruses. EUBANKS CARSNER, Chm., C. W. BENNETT, MAX W. GARDNER, F. O. HOLMES, JAMES JOHNSON, H. H. MCKINNEY, H. H. THORNBERRY, FREEMAN WEISS.

Phytopathological Classics. H. H. WHETZEL, Manager, H. B. HUMPHREY, Editor.

Public Relations. J. A. PINCKARD, Chm., O. C. BOYD, C. T. GREGORY, J. H. JENSEN, FRANK MCWHORTER, A. G. NEWHALL, G. H. STARR, A. J. ULLSTRUP, G. F. WEBER, P. A. YOUNG.

Regulatory Work and Foreign Plant Diseases. J. F. ADAMS, Chm., A. A. BITANCOURT, A. B. BUCHHOLZ, S. J. P. CHILTON, F. L. DRAYTON, M. R. HARRIS, W. A. MCCUBBIN.

Standardization of Fungicidal Tests. S. E. A. MCCALLAN, Chm., J. G. HORSFALL, KENNETH KADOW, R. W. LEUKEL, J. W. ROBERTS, FRANK WILCOXON, J. D. WILSON.

TEMPORARY COMMITTEES

Auditing, V. F. TAPKE, H. A. RODENHISER.
Elections, JOHN W. HEUBERGER, ROBERT O. MAGIE, H. W. THURSTON, JR.
Resolutions, M. F. BARRUS, ANNA E. JENKINS, N. J. GIDDINGS.

REPORTS OF OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1940

Report of the Secretary. The Society year 1940 opened with 1082 members and closed with 1128, a gain of 46 members. At the Philadelphia meeting 84 new members were elected. Nine former members were restored to the active roll during the year. The Society lost 47 members, 14 by resignation, 5 by death, and 28 by suspension for nonpayment of dues. Of the full membership, 154 are paid-up life members, and 4 are paying $10.00 a year toward life membership.

The Society's clearing agency, to facilitate contact between employable plant pathologists and pathological openings from individuals or institutions, was continued in 1940. Applications were received or on hand from 45 pathologists. Sixty-one applications from 41 pathologists were sent to 9 prospective employers. It was reported to the agency that one pathologist was hired through contact first made through the agency.

Report of the Treasurer. Statement of accounts for the year ending November 30, 1940.

Receipts:

Balance from 1939				$5185.89
1936	$ 10.00	($10.00 life)		
1937	10.00	(10.00 life)		
1938	20.00	(20.00 life)		
1939	47.44	(20.24 life)		
1940	2817.56	(30.00 life)		
1941	1716.40	(10.00 life)		
1942	1.50		$4622.90	
Interest on savings account			12.72	
Items for other accounts included in checks for dues:				
Sales	1.50			
Classics	4.00			
Memoir	0.50		6.00	
Balance from A. P. S. dinner in Columbus			7.13	
To replace checks returned by bank			13.60	
Total receipts				4662.35
				$9848.24

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY:			
1936–37	8.00		
1938	8.65		
1939	2683.36		
1940	3623.80	$6323.81	
Transferred to Sinking Fund (Building and Loan)		75.00	
Transferred to PHYTOPATHOLOGY for publication of Society material		577.38	
Secretarial work for Secretary and Treasurer		444.25	
Expenses of office of Secretary		48.93	
Expenses of membership committee		7.92	
Preprints of abstracts		40.39	
Printing		139.37	
Stamps and envelopes		8.62	
Supplies and furniture		50.65	
Transferred to PHYTOPATHOLOGY for sales		1.50	
Transferred to Classics		4.00	
Transferred to E. F. Smith Memoir account		0.50	
Expenses of Columbus dinner (balance)		7.13	
Collection charges on checks		0.90	
Telegram		0.58	
Checks returned by bank		13.60	
Total expenditures			7744.53
Balance on hand			2103.71
			$9828.24

(Note: During the year $2600.80 was transferred from the savings account in the Morris Plan Bank to the Society's checking account.)

Sinking Fund. The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, is obtained by deducting $5.00 from each life-sustaining membership installment. This fund totaled $9571.00 at the close of 1939. During the year ending November 30, 1940, it increased to $9646.00 and is invested as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection ($1000.00 at 6%, $500.00 at 5%)	$1500.00
Invested with the following building and loan associations:	
Arlington and Fairfax Bldg. and Loan, 5%	1000.00
Columbia Permanent Bldg. Association, 4%	530.60
District Bldg. and Loan Association, 4%	1591.80
National Permanent Bldg. Association, 4½%	1045.65
Northwestern Federal Savings and Loan Association, 4%	2000.00
Perpetual Bldg. Association, 4%	1061.20
Prudential Bldg. Association, 3½% (accrued interest $55.64)	1201.64
	$9930.89
Less interest due PHYTOPATHOLOGY (accrued, 1940, $207.67)	284.89
	$9646.00

The Lyman Memorial Fund, obtained from voluntary contributions, now totals $3264.66, of which $172.59 represents accrued interest and is available for PHYTOPATHOLOGY. The whole amount is invested with the Brookland Building and Loan Association at 3½%. The account for 1940 follows:

Balance on hand, Dec. 9, 1939	$3003.00
Dividends, Dec. 31, 1939, and June 30, 1940	115.03
Contributions from members	46.00
Sale of Erwin F. Smith Memoir	100.63
Balance, December, 1940	$3264.66

Report of the Business Manager of Phytopathology. At the close of 1940 there were 600 nonmember subscribers on our mailing list, including 5 complimentary. This compares with 651 reported last December. During the year there were 67 cancellations and 57 suspensions for nonpayment, a loss of 124. In spite of 73 new paid subscriptions, there is a net loss of 51, compared with a net gain of 13 last year and 23 in 1938. It is to be expected that there will again be a loss for 1941, as the present European list of 160 will no doubt decrease considerably. The most noticeable changes for this past year were a drop in German subscriptions from 25 to 1 and in subscriptions from the U.S.S.R. from 89 in 1939 to 51 in 1940. Japan's list, however, remained unchanged at 61, while there was a small increase in paid subscriptions to China, from 9 to 13. It is rather surprising to note that, before December 15, we had already received payment for 7 renewals of England's 35 subscriptions and 2 of Scotland's 3, and that orders had been received at this time for 24 subscriptions to the U.S.S.R. as compared with 51 during the entire year just past.

Statement of accounts for the year ending November 30, 1940.

Receipts:

Balance from 1939			$ 2967.87
Subscriptions:			
1938	$ 5.85		
1939	70.35		
1940	3417.14		
1941	328.07		
1942	5.50	$3826.91	
Member subscriptions:			
1936–37–38	16.65		
1939	2683.36		
1940	3623.80	6323.81	
Sales of back numbers		290.25	
Advertising:			
1939	190.86		
1940	675.28	866.14	
Interest on Sinking Fund:			
First mortgage notes	85.00		

Building and Loan	162.72	247.72	
Grant from Rockefeller Institute		600.00	
Interest on savings account		125.37	
From Society for publication of A. P. S. material		577.38	
Allowance on reprints		606.59	
Payment by authors for excess illustrations		201.97	
Sale of periodicals		10.85	
Draft redeposited		188.50	13865.49
			$16833.36

Additional interest of $207.67 was allowed to remain with the various Building and Loan associations. Also interest of $115.03 was left with the Brookland Building and Loan Association (Lyman Fund).

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY (11 numbers)

Vol. XXIX, No. 12 and Index	$818.53			
Vol. XXX, No. 1	767.55			
No. 2	784.50			
No. 3	743.34			
No. 4	882.59			
No. 5	603.72			
No. 6	701.83			
No. 7	634.38			
No. 8	663.23			
No. 9	654.89			
No. 10	642.04	$7896.60		
Postage		518.77	$8415.37	
Secretarial work and office expenses, Editor in Chief			374.80	
Secretarial work, Business Manager			252.50	
Allowance and expenses, Advertising Manager, 1940			87.14	
Commission, Advertising Manager, 1939			52.15	
Stamps and stamped envelopes			89.02	
Supplies and furniture			24.95	
Printing			8.69	
Advanced for Cumulated Index account			19.16	
Refunds, subscriptions and sales			17.15	
Import tariff, Canada			0.63	
Service charge, checking account			0.70	
Checks returned by bank			202.75	
Total expenditures				$ 9545.01
Balance on hand:				
Checking account			$2713.35	
Northwestern Fed. Sav's and Loan Association			4575.00	7288.35
				$16833.36

(Note: During the year $2575.37 was transferred from the savings account in the McLachlen Banking Corporation to the checking account and $4500 transferred from there to a new account with the Northwestern Savings and Loan Association.)

Report of the Auditing Committee for the year ending November 30, 1940. The books of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY have been examined, together with the present investments of the Sinking Fund and the Lyman Memorial Fund. The accounts have been found correct and the books are in excellent order.

V. F. TAPKE
H. A. RODENHISER

December 19, 1940.

Report of the Advertising Manager. Contracts in 1940 totaled $870.86. There were 103 revenue-producing advertisements, occupying 53½ pages and consisting of 23 full-page, 42 half-page and 38 quarter-page insertions. During the year 14 commercial firms used our journal as an advertising medium.

There were 46 nonrevenue-producing advertisements, occupying 37¼ pages, made up of notices regarding Phytopathological Classics, the Society clearing agency for employment of plant pathologists, the Committee on Publicity, the Erwin F. Smith Memoirs and also several complimentary and exchange ads.

On account of its specialized field and its resultant comparatively small mailing list (not to mention the fact that our members apparently do not mention PHYTOPATHOLOGY when writing to advertisers), the advertising cannot be made to cover the cost of publication, as is the case with journals of large circulation, but it is believed that, with the cooperation of the membership of the Society, it might be increased sufficiently to pay for approximately two numbers per year. This would mean contracts totaling at least $2000.00, or about 125 pages, considerably more than has ever been carried.

AGNES E. MEIER

Report of the Editor in Chief. Volume 30 of PHYTOPATHOLOGY contains, exclusive of the index, 1056 pages of printed matter and illustrations, classified as follows: One hundred seven articles, 44 notes, 3 reports of meetings, 9 book reviews, 189 abstracts (1 by title only), 251 text figures and 2 plates. From Dec. 19, 1939, to Dec. 15, 1940, a total of 195 manuscripts of articles, notes, book reviews, and reports of Society meetings were submitted for publication in our Journal. Five manuscripts were withdrawn and 6 were returned to their contributors as unacceptable. There are now in press 44 articles and 121 abstracts. Other manuscripts now (Dec. 15) on hand number 50, or a total of 580 typewritten pages.

It is quite generally conceded that PHYTOPATHOLOGY takes rank with the better scientific journals of the world. Even so, there is room for improvement. I cannot overemphasize the necessity of still greater devotion to the matter of manuscript preparation. Scarcely an article comes to us that could not have been materially condensed without impairment of scientific or literary worth. Many of these, after review and criticism by one or more editors, are returned to their authors once, twice, even three times, before they are acceptable.

Too much attention cannot be given to the proper and accurate listing of citations to literature. The same applies also to setting up tabular matter. Too many contributors still leave to the editor the details of table construction, even to the extent of expecting him to compose suitable table headings. To be sure such carelessness is the exception rather than the rule, but such exceptions prove the rule and try the patience of those who must pass final judgment upon the manuscripts.

Grateful acknowledgment is here made to Dr. F. V. Rand for his preparation of the index and to all others who have contributed editorially and otherwise to the excellence of our Journal.

H. B. HUMPHREY

Report of the Manager of Phytopathological Classics for the year 1940. I beg to submit herewith the annual report of my stewardship as Manager of Phytopathological Classics:

Record of Copies Sold and on Hand

	On hand December 1, 1939	Sold during year	On hand December 1, 1940
Classic #1	92	12	80
#2	293	12	281
#3	392	12	380
#4	454	12	442
#5	702	24	678
#6	838	25	813

Cash balance on hand 12-1-40	$225.49	
Receipts during year	60.25	
Total		$285.74
Expenditures:		
Postage	$ 3.25	
Total expenditures		3.25
Balance on hand December 1, 1940		$282.49
Due on accounts		5.50

H. H. WHETZEL

Report of the Necrology Committee. *Deaths previously unreported:*

GUSTAV KÖCK, July 28, 1939, and
HUGO KOBLENZER, Dec. 2, 1939.

Deaths during 1940:

HAVEN METCALF, May 23;
ORMUND ROURKE BUTLER, Oct. 24; and
WILLIAM WILLIAMS GILBERT, Dec. 22.

A. G. JOHNSON
M. B. WAITE

Report of the Committee on Biological Abstracts and the Union of American Biological Societies. This report was published in PHYTOPATHOLOGY 31: 199. 1941.

Report of Extension Work and Relations Committee. The Extension Work and Relations Committee sponsored two activities in 1940: The first was a conference on "Recent Studies on Forage Crop Diseases," with special emphasis on diseases of (1) alfalfa, (2) Austrian winter peas and vetch, and (3) grasses. The principal discussion leaders were Dr. J. L. Weimer, Dr. John Monteith, and Mr. Lawrence Henson. This conference was held on the afternoon of December 28 at the Philadelphia meeting. A detailed report of this conference will appear in the Extension Plant Pathologist in the near future. The attendance at this conference was about 100.

The second activity was a conference on "The Place of the Plant Pathologist in the National Defense Program." This conference was held on the evening of December 30 at the Philadelphia meeting. The general opinion expressed at this conference was that the war situation has not yet created an emergency in the field of plant pathology but has attached greater significance to disease control as a means of more economical production of crops.

Several people in attendance at this conference showed color slides on a variety of subjects. The object of this exhibition was to develop a system of slide exchange and duplication among those interested.

LUTHER SHAW, Chairman, C. C. ALLISON, R. J. HASKELL, G. W. KEITT,
R. H. PORTER, O. A. REINKING, R. C. ROSE, D. R. SANDS, W. B. TISDALE.

Report of the Committee on Coordination in Cereal and Vegetable Seed Treatment Research. *Cereal Seed Treatments.* The experiments set up and carried out in 1939 were repeated practically unchanged in 1940. An experiment to determine the response of flax to treatment was added, however. Briefly the experiments are of two types: a disease control test and a yield test. In the disease control test heavily smut-inoculated seed of susceptible varieties of wheat and oats, and stripe-infected barley seed were treated with 5 to 7 different disinfectants and grown at widely separated locations to determine the effects of environment on the effectiveness of these disinfectants in controlling disease and increasing seedling stand. On the other hand, in the yield tests only commercial varieties of wheat, oats, barley, and flax were treated with a smaller number of disinfectants and were grown at some of the same stations. In all, 13 different State and Dominion experiment stations cooperated, 16 sets of seed were sent out for the disease-control tests and 13 sets for the yield tests. To date, complete sets of data have been received from 5 of the stations, and it is expected that results will be received from the others soon.

The data from the 1939 tests have been tabulated and summarized but have not been mimeographed nor distributed. In the course of the next few months it is planned to combine the results of the 2 years' work and distribute them to the cooperators, and if it seems advisable, to publish a summary of the work thus far.

Vegetable Seed Treatments. This year, for the first time, active experimental work was undertaken in the testing of treatments for vegetable seeds. Under the direction and through the efforts of Harold T. Cook of the Virginia Truck Experiment Station, 31 sets of uniform seed-treatment tests were conducted by cooperators in 18 States to determine the relative value of red copper oxide, zinc oxide, and Semesan for improving the seedling stands of beets, cabbage, cucumbers, peas, spinach, and tomatoes. Results from all of the 31 tests have been compiled, analyzed statistically, and mimeographed for distribution to the collaborators. Quoting from Dr. Cook's report, "A number of rather striking facts are brought out by the cooperative tests in 1940, which, because of the uniform methods used, the wide range of environmental conditions encountered, and the statistical significance of the data, should serve as a reliable basis on which general recommendations may be made for treating seed of the crops included in these tests."

Recommendations of the Committee. 1. That the cereal and vegetable seed treatment programs be continued. 2. That the Council or the Society consider the advisability of setting up the vegetable seed-treatment work under a separate committee to include the member of the present committee who is primarily concerned with vegetables. It is desirable to increase the number of vegetable pathologists in order better to spread the very considerable burden and expense of the vegetable program in its present extensive form, and, since experimental programs in cereal and vegetable seed-treatments involve widely different fields as regards crops, disinfectants, geographic areas, and scientific workers, it seems probable that it would be best to organize a separate committee to carry on the vegetable work.

Report of the Committee on Standardization of Fungicidal Tests. The Committee on Standardization of Fungicidal Tests is cooperating with the American Association of Economic Entomologists Committee on Insecticide Terminology. Several informal meetings between individual numbers of the two committees have been held, and a joint meeting of the two groups is planned during the Philadelphia convention. The aims of the two committees are essentially alike and their cooperation will add materially to the usefulness of each.

Attention has been continued on A—Laboratory methods, B—Field methods, and C—Laws regulating the sale of fungicides.

A—Laboratory methods. Several original papers have been published on this subject during the current year, others are known to be in manuscript. In addition certain fundamental studies are in progress. Because of this recent activity it is felt desirable to postpone the revision of the tentative methods prepared in 1939. Copies of these tentative mimeographed methods may be still obtained. They are: (1) A Standard Bordeaux mixture for laboratory tests and for determination of Bordeaux coefficient; (2) Determination of mean particle diameter of fungicides; (3) Specifications for slide-moist-chamber method of testing protective fungicides.

At present the greatest need in the development of laboratory methods is the correlation of laboratory and field results, so that the proper emphasis may be laid on the various laboratory procedures. To this end, cooperative laboratory and field tests on fruits and vegetables are being carried on in four States.

B—Field methods. The tentative recommendation on standard spray nomenclature is being revised with respect to terms defining bud development in cooperation with the Entomologists committee.

It is recommended that the Society express all spray materials in standard units of weight (avoirdupois) or liquid measure (U. S.) sufficient to make 100 gallons of spray, and that all dust formulae be stated on a basis of 100-pounds total mixture. It is further suggested that PHYTOPATHOLOGY adopt such a nomenclature, and that the members of the Society seek uniform adoption of this usage in the publications of institutions with which they are connected.

Cooperative tests on efficient designs for comparing fungicidal treatments in field experiments are in progress in four States.

C—Laws regulating the sale of fungicides. The sub-committee, Dr. John W. Roberts and Dr. Errett Wallace, delegated to study this question, is of the opinion that no further action be taken at this time; the committee as a whole is in agreement.

Your committee wishes to express its approval of the Council's policy of rotating the membership of its Standing Committees and thereby increasing their activity and usefulness.

S. E. A. McCALLAN, Chairman, J. W. ROBERTS, H. C. YOUNG,
K. J. KADOW, J. G. HORSFALL, C. E. YARWOOD, R. H. DAINES

Report of the Advisory Committee for 1940. A number of suggestions was submitted during the year to the Advisory Committee on programs and society activities, by its members, which were given attention. The opinions on these suggestions have been sounded out by the Committee among representative centers of the Society. In general, as in the past, the survey indicated that there was a satisfaction with the programs as they are conducted at present.

The following matters have been considered by the Committee and are being suggested to the Council. Although there was not a complete agreement on all of the matters dealt with, it was fairly uniform on the following suggestions:

1. The self-introduction of the membership at one of the business meetings should not be attempted because of the lack of time. Lapel identification should be followed.
2. The president's address at the annual meetings should not be revived.
3. That only local talent of the Society and not outside professional entertainers should be used for the entertainment following the annual banquet. Occasional use of some outside talent should be allowed only when there seems to be no practical way of handling the matter in the traditional way.
4. A slight majority was of the opinion that there should be a more critical selection by the Editorial Committee of papers given at the meetings.
5. A similar majority favored the enlargement of the round-table discussions if they were kept specific enough. A period of "mutual aid" designed for members to present personal difficulties encountered in their work in the hope that some one in the group might offer assistance met with disfavor.
6. From a questionnaire circulated by the Committee among a small but representative portion of the Society, a considerable majority favored a revision of the method of selecting officers by the appointment of a nominating committee. A slight majority favored the Council appointing this committee, although many favored that it be appointed by the President or elected by the membership.
7. By a considerable majority, it was proposed that the Council not be enlarged, even though the membership has greatly increased in recent years.

Respectfully submitted,

W. J. ZAUMEYER, Chairman, F. L. DRAYTON, A. A. DUNLAP,
J. A. PINCKARD, R. K. VOORHEES, J. C. WALKER, C. E. YARWOOD

Report of the Committee on Nomenclature and Classification of Plant Viruses. The American Committee on Nomenclature and Classification of Plant Viruses was appointed by the President of The American Phytopathological Society at the 1938 meetings at Richmond and has now been requested by the Secretary to make its first report. When the Committee was organized its purpose was, in part, at least, to cooperate and coordinate its work with that of the International Committee on Virus Nomenclature appointed at the Fifth International Botanical Congress meeting in Cambridge, England, in 1930. The first meeting of the International Committee was at Amsterdam in 1935, and the last at New York in September, 1939. With the outbreak of war, the International Committee practically ceased to function, and it appeared desirable to some members of the American Committee that it should continue the project more or less independently of the International Committee, hoping to renew cooperation or agreement when international avenues for coordination again become available.

The original purpose of both committees was to secure an early universal agreement on virus nomenclature in order to reduce the confusion in nomenclature already in existence and especially to prevent new and added confusion through the introduction of individual or independent systems of virus nomenclature. However, the work of the committees has apparently stimulated interest in virus nomenclature to the extent that several independent systems of nomenclature have already been proposed or are in preparation. A majority of the American Committee members, past or present, is of the opinion that the several systems should be given a trial or test in published form so that the best one may eventually be selected. A policy of haste has been repeatedly urged upon both the International and the American Committees, but this Committee feels that the present time is not propitious to make definite recommendations.

Sub-committees have been organized covering the principal crop divisions such as cereals, fruits, vegetables, potatoes, tobacco, and ornamentals to assist in compiling the synonyms and descriptions of the viruses associated with these plant groups.

The Committee has approved a proposal of The Plant Disease Survey to publish a list of virus synonyms to be prepared with the assistance of this Committee.

Submitted with the approval of the Committee, December 29, 1940.

JAMES JOHNSON, Chairman

Report of the Committee on Publicity and Public Relations. At the annual meeting of the Society in 1939 the temporary Committee on Publicity and Public Relations was made permanent. The function and suggested policies of the Committee were outlined in a report to the Society at that time.

During the past year the Committee has attempted to follow these policies in preparing approximately 255 news stories of which 130 were on subjects discussed at the Philadelphia meeting this year. The Committee has not attempted to report on every article appearing in PHYTOPATHOLOGY, since it has exercised its own judgment with respect to the news-worthiness of each technical article brought to its attention. These news releases have been prepared carefully and with utmost caution in order that only factual releases would find their way into responsible news channels. Every attempt has been made to route each worthy press release into those channels serving that section of the country most intimately concerned with the subject covered.

Your committee is unable to report on the total numbers of articles actually used by the press. Neither is it able to report upon the quality of the ultimate product, since clippings are not returned to us except by some member of the society who happens to clip the article from his local paper. Many of our releases have been found in *Science*, *Science News Letter*, the national wire services, and in certain farm and trade journals.

The Committee wishes to express its gratitude to the Editor in Chief of PHYTOPATHOLOGY, H. B. Humphrey, and to our printer, George M. Houck, Science Press Printing Co., for their prompt and regular contribution of page proofs of PHYTOPATHOLOGY. The Committee also wishes to express its thanks to Howard W. Blakeslee, science editor of the Associated Press, Frank Thone, Science Service, and to the several members of the Association of Science Writers for their kind assistance.

C. T. GREGORY, Chairman, O. C. BOYD, J. H. JENSEN, J. A. PINCKARD, G. H. STARR, FRANK MCWHORTER, A. G. NEWHALL, A. J. ULLSTRUP, G. F. WEBER, P. A. YOUNG

Report of Committee on 30-Volume Index of Phytopathology. Your Committee's report this time will be as short and to the point as possible. As of December 15, complete material has been received from the indexers for all volumes except 3, 4, 14, 18, and 25. The indexing sheets have been checked against the original papers, given the first editing, thrown into the individual entries, and alphabetized to the first letters for 18 volumes. There are 12 yet to be given the final alphabetizing, editing, and preparation of manuscript for the printer.

Respectfully submitted,

FREDERICK V. RAND, Chairman

Resolutions of the Committee on Resolutions. RESOLVED that The American Phytopathological Society express its appreciation to the American Association for the Advancement of Science for the satisfactory arrangement made by members of its committees for the meetings of the various sessions of our Society.

RESOLVED that The American Phytopathological Society convey its appreciation to the management of the Hotel Philadelphian, and especially to Earle W. Baker, assistant manager, for unstintingly providing our officers and committees with rooms for conducting business and for general services to our members.

RESOLVED that this Society express its appreciation to the committee on local arrangements, P. P. Pirone, chairman, L. M. Black, Edgar Rex, L. W. R. Jackson, B. H. Davis, and O. D. Burke, for selecting satisfactory meeting places and comfortable quarters for our members; to the Franklin Institute for providing projection apparatus; to the banquet committee, H. W. Thurston, Jr., chairman, K. J. Kadow, P. D. Peterson, and St. John P. Chilton, and to others who assisted in various ways, for the splendid entertainment that made the annual dinner so enjoyable; and to the committee on demonstration sessions on Sunday afternoon, which provided a very instructive feature of our meetings.

RESOLVED that this Society commend the program committee for assigning to the same headquarters The Mycological Society of America and this Society, thereby facilitating conferences of workers in related fields of research and further strengthening the ties between the two societies.

RESOLVED that this Society on behalf of its members express to the officers and members of committees our sincere appreciation for the efforts they have made to promote the interests of the Society throughout the year and to make this annual meeting instructive and enjoyable.

Respectfully submitted,
M. F. BARRUS, Chairman
N. J. GIDDINGS
ANNA E. JENKINS

ACTION BY THE SOCIETY AT THE 1940 PHILADELPHIA MEETING

Elections and Appointments. The appointments made, as provided by the Constitution, by the President or the Council since the previous meeting, were approved by the Society in business session. The election committee opened and counted the ballots, and the results were announced to the Society at the banquet. The names of those elected and appointed appear earlier in this report in the list of officers, representatives, and committees. Eighty-five applicants were elected to membership.

The Society confirmed the Council's appointment of the following new members to the Editorial Board of PHYTOPATHOLOGY: G. M. Armstrong, T. C. Vanterpool, Alma M. Waterman, B. L. Richards, and S. P. Doolittle.

Reports of Officers, Representatives, and Committees. The reports for the year 1940, as presented on previous pages, were read and accepted.

Recommendation Regarding Abstracts. The Society confirmed the Council's recommendation that Standing Rule 3(a) contain the additional sentence: "The Secretary shall return to authors all abstracts received after the closing date."

Recommendation Regarding "Staggering" of Terms of Office of Secretary, Treasurer, and Editor in Chief of PHYTOPATHOLOGY. The Society confirmed the Council's recommendation that appointments for the offices of Treasurer and Business Manager of PHYTOPATHOLOGY, Secretary, and Editor in Chief of PHYTOPATHOLOGY be made now for three-year terms, but in order to accomplish constitutionally the wish of the members (which the Council believes to be to the interest of the Society), it has been arranged that resignations will be presented by two of the appointees, that of the Secretary at the close of 1941 and that of the Editor in Chief at the close of 1942, so that the terms of office may be "staggered."

Committee on Nomenclature and Classification of Plant Viruses. The Society confirmed the Council's recommendation that, in view of the request for rotating committees, the Council will plan to rotate the chairmanship of this committee each year.

Committee on Regulatory Work and Foreign Plant Diseases. The Society confirmed the Council's appointment of a standing committee on Regulatory Work and Foreign Plant Diseases.

Committee on Public Relations. The Society confirmed the Council's recommendation that the standing committee on Publicity and Public Relations be henceforth designated as the Committee on Public Relations.

Temporary Committee on Fungous Nomenclature. The Society confirmed the Council's recommendation that the temporary committee on Fungous Nomenclature to Work with the British Section of the Mycological Society be continued as a temporary committee until the revision of the committee system, after which time it will become a special committee.

Committee Reports. The Society confirmed the following recommendations of the Council concerning committee reports: (1) that only the reports of standing committees be published in the annual report without authorization of the Society; (2) that the personnel of committees be printed only in the annual report and in the program of the annual meeting, with a footnote to be printed in PHYTOPATHOLOGY indicating in which issue the list will be found; (3) that the Secretary request chairmen to make their reports as brief as possible.

Revision of Constitution. The Society confirmed the Council's recommendation that a committee be appointed to re-word Article VII, with the objective of revising the committee system, to read: ''Temporary, Special, or Standing Committees.'' Standing committees are to be appointed by the Council to take care of matters pertaining to Society relationships. Special committees are to be appointed by the Council to do specific problems in plant pathological work. Temporary committees are to be appointed by the President.

Demonstration Session. The Society confirmed the Council's recommendation to the Program Committee for the continuance of the demonstration session on an invitational basis, the number of demonstrations to be determined by space available and other circumstances.

New England Division. The Society confirmed the Council's recommendation of the recognition of the New England Division of The American Phytopathological Society, and thereby authorize such group to have one representative to the Council.

Pledge of Support to National Defense Council. The Society confirmed the following recommendation of the Council: ''The American Phytopathological Society and Council pledge support to the National Defense Council and authorize the officers and Council to serve the National Defense Committee in any capacity that may be necessary.''

Foreign Relief Subscription. The Society passed the motion that the Editor and Business Manager of PHYTOPATHOLOGY be authorized to distribute a blank form to the domestic subscribers to PHYTOPATHOLOGY, with the January number, providing for receiving subscriptions to a foreign relief subscription fund.

Banquet Proceeds. The Society confirmed the Council's recommendation that any proceeds made from a banquet be maintained in a separate fund for that purpose and not be diverted except by special action of the Council.

Summer Meeting. The Society, on Council recommendation, voted to hold a summer meeting, at the invitation of the New England Group, in New Haven, Connecticut, June 23–27, 1941.

The 1941 Annual Meeting. It was recommended by the Council, and voted by the Society, that the annual meeting in 1941 be held at Dallas, Texas, December 29, 1941, to January 2, 1942, and that it be a hotel meeting.

MEMBERS OF THE TOBACCO DISEASE AND THE PLANT NEMATODE COUNCILS TO HOLD A JOINT SUMMER MEETING

Plans are now being made for a joint meeting of the Tobacco Disease Council and the Plant Nematode Council to be held at the Georgia Coastal Experiment Station, Tifton, Georgia. Probable dates are June 24, 25, and 26. The program will consist of topics on both tobacco-disease and nematode problems, as well as of trips to the experimental plots where much disease and nematode research is being conducted.

The Tobacco Disease Council was organized in 1935, by a group of Federal and State workers who met at Greensboro, N. C., on November 5 and 6 of that year. Membership is open to all research and extension workers on tobacco-disease problems in the United States, and the purpose of the Council is to promote cooperation in an energetic campaign of scientific investigation leading to effective tobacco-disease control. Since its organization, the Tobacco Disease Council has met annually with an average attendance of about 50 members. At these meetings the research workers make progress reports, and discuss disease-control recommendations; whereas the extension pathologists discuss results obtained during the year with disease-control practices and point out flaws in current recommendations for the control of tobacco diseases.

The Plant Nematode Council was organized under the name "Root-Knot Nematode Committee" by Federal and State workers at Nashville, Tennessee, on February 2, 1937. At the third National Plant Nematode Conference, held at Birmingham, Alabama, February 9, 1940, the name of the organization was changed to "Plant Nematode Council." Its purpose is to promote research on plant nematode problems and to facilitate mutual assistance among the workers. Membership in the Plant Nematode Council is open to all who are interested in plant nematode research.

H. H. Hume, Agricultural Experiment Station, Gainesville, Florida, is Chairman and H. P. Barss, Office of Experiment Stations, Washington, D. C., is Secretary of the Plant Nematode Council. S. A. Wingard and R. G. Henderson, of the Virginia Agricultural Experiment Station, Blacksburg, Virginia, are Chairman and Secretary, respectively, of the Tobacco Disease Council.

S. A. WINGARD, *Chairman*
Tobacco Disease Council

ORMOND ROURKE BUTLER

August 14, 1877–October 24, 1940

Dr. Butler received the Diploma of the Institut Nationale Agricole, Switzerland, in 1895. He was graduated from the University of California with the degree of Bachelor of Science and received the degree of Master of Science from the same institution in 1905. He was granted the degree of Doctor of Philosophy by Cornell University in 1910.

From 1895, he was viticulturist-horticulturist in California; from 1910 to 1912, he was research instructor in horticulture at the University of Wisconsin; and from 1912 to the time of his death, he was head of the Department of Botany at the University of New Hampshire and Botanist of the New Hampshire Agricultural Experiment Station.

Dr. Butler, in addition to being a member of The American Phytopathological Society, was a member of the American Society of Agronomy, fellow of the American Association for the Advancement of Science, member of Sigma Xi and Phi Kappa Phi.

In his chosen field Dr. Butler was one of the world authorities on copper compounds in relation to control of fungous diseases. His published papers on copper fungicides are classics. Dr. Butler gave special attention to the control of diseases of apples and potatoes and to the influence of environmental factors on mosaic symptoms. He was the moving spirit in building up the growing of seed potatoes in New Hampshire and in the present advanced practice of potato seed certification.

WILLIAM WILLIAMS GILBERT

September 17, 1880–December 22, 1940

William Williams Gilbert was graduated from the University of Vermont in 1904 with the degree of Bachelor of Science in Agriculture, and in 1909 he received the degree of Master of Science from the same institution. He also pursued graduate study at the University of Wisconsin in 1912, at George Washington University, 1913–14; and at Columbia University, 1914–15. He was elected to Phi Beta Kappa at the University of Vermont.

Mr. Gilbert was Assistant in Botany at the University of Vermont, 1903–04; and from 1904 to the time of his death he served in various capacities in the Bureau of Plant Industry, U. S. Department of Agriculture, as follows: Special Agent, 1904–05; Scientific Assistant, 1905–08; Assistant Pathologist, 1908–12; Pathologist, 1913–28; Senior Pathologist, 1928 to the time of his death.

He was a sustaining life member of The American Phytopathological Society.

Mr. Gilbert did notable work on the diseases of cotton and vegetable crops. In the later years, he did editorial work on scientific manuscripts, for which he was unusually well qualified both by training and disposition. His congenial personality endeared him to all his associates. His quiet yet jovial manner, together with sincerity and absolute integrity, made him a friend that is greatly missed.

HAVEN METCALF

August 6, 1875–May 23, 1940

Haven Metcalf attended Colby College and Brown University, was graduated from the latter in 1896 with the degree of Bachelor of Arts and was granted the degree of Master of Arts by the same institution in 1897. He continued his studies at Harvard University in 1899, at Indiana in 1900, and at the University of Nebraska where he received the degree of Ph.D. in 1903.

From 1896 to 1899 he was Instructor in Botany at Brown University; 1899 to 1901, Professor of Biology at Tabor College; 1901 to 1902, Instructor in Bacteriology at Nebraska; 1902 to 1906, Associate Professor of Botany and Bacteriology at Clemson College; 1904 and 1905, Special Agent of the United States Department of Agriculture; in 1906, he became Pathologist in the Bureau of Plant Industry of the United States Department of Agriculture and in 1907 he organized and became the head of the Laboratory of Forest Pathology. In this last position he remained till his death in 1940, when he was Principal Pathologist in charge of the Division of Forest Pathology.

Dr. Metcalf was a sustaining life member of The American Phytopathological Society and its President in 1914. He was a member of numerous other scientific societies.

Although early in his career Dr. Metcalf did very creditable work as a teacher and in research in bacteriological problems and rice diseases, his great life work was the development of the science of forest pathology in the United States and especially of forest pathology research. He was the inspiring leader in this line for 33 years. He was not only himself a splendid investigator, but he had great ability in organizing research and in bringing together in harmonious cooperation the parties interested in these problems. This was especially revealed in his organization of the investigation of the problems presented by the great epidemic tree diseases that have invaded America, the chestnut blight, the white pine blister rust, and the Dutch elm disease. He was noted for intellectual honesty, thoroughness in research and organizing ability. He was a man of charm, interested in art, music, philosophy and literature, as well as in science in its broadest sense.

ERRATA, VOLUME 30

Page 257, citation 2 of Literature Cited *read* Phytopath. **30**: 171–174. 1940 *for* (In press).

Page 260, table 1, column 4 *read* 94 *for* 92.

Page 296, *Add* as footnote to table 6, [16] See footnote 8.

Page 297, citation 4, of Literature Cited, *read* Bailey *for* Baley.

Page 455, line 52, *read Urocystis for Ustilago.*

Page 580, line 5, *read* plain *for* plant.

Page 836, line 15, *read* loss *for* less.

Page 977, table 1, column 8 *read* in to *for* into.

Add author names to index: BERCH W. HENRY, p. 1047. ELIZABETH C. WAGNER, p. (1047).

FURTHER EXPERIMENTS ON THE CONTROL BY SEED DISINFECTION OF ROOT-ROTTING FUNGI IN WHEAT[1]

J. E. MACHACEK AND F. J. GREANEY[2, 3]

(Accepted for publication September 16, 1940)

INTRODUCTION

Seedling blight of wheat, in Manitoba usually caused by the root-rotting fungi *Helminthosporium sativum* P., K., and B. and *Fusarium culmorum* (W. G. Sm.) Sacc., has been shown by the writers (2, 7, 8) to be controllable by proper seed disinfection. Organic mercury compounds gave the greatest degree of control, but usually, even with these materials, the beneficial effects of seed treatment were not sustained during the entire life of the plant. Thus, although in certain years the stand of plants was considerably improved by seed treatment, the improvement was not reflected by increased crop yields.

In the present investigations, a special effort was made to discover the reason for the inconsistency of the yield results obtained in the previous experiments. In addition, 9 untried and unnamed seed disinfectants and 4 untried commercial seed disinfectants were studied, the joint effect of seed disinfection and soil fertilization was measured, and an attempt was made to determine the effect or organic mercury dusts on disease-free wheat seed. The results of these studies, carried on from 1935 to 1939, inclusive, are presented and discussed in this paper.

EXPERIMENTAL METHODS

All of the experiments described here were performed at Winnipeg, and to compare the Winnipeg results with those obtained under other conditions of soil and climate, supplementary tests were made at other points in Manitoba; namely, at Souris, Treherne, and Morden.

In 1935 and 1936, a number of untried, unnamed dusts, were subjected to field tests in which their fungicidal efficiency was compared with that of New Improved Ceresan. The dusts were applied, according to the requirements of the experiment, to wheat seed that was previously infested with spores of *Fusarium culmorum.* The wheat variety Mindum was used in 1935 and the variety Renown in 1936.

About one day after the seed treatments were completed, the seed was sown in the plots by hand or with a V-belt hand drill. At Winnipeg, inoculum of *Fusarium culmorum* was added to the rows as the seed was planted. This inoculum, added to the soil at the rate of 600 cu. cm. to a rod

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[1] Contribution No. 637 Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Assistant Plant Pathologist and Associate Plant Pathologist, respectively.

[3] The writers wish to thank F. W. Berk and Co., Ltd., London, England; McKenzie-Stephenson, Ltd., Winnipeg, Manitoba; and Canadian Industries, Ltd., Winnipeg, for their kindness in providing the fungicidal materials used in this investigation.

[VOLUME 31, NUMBER 5, MAY 1941]

row, was prepared by mixing a culture of the fungus with autoclaved soil. The fungus was grown on autoclaved ground oat hulls and then mixed with soil in the proportions of 1 part of culture, by volume, to 9 parts of soil. After an incubation period of about 2 weeks, the mixture was ready for use. For a supplementary test at Souris in 1936, the seed was not artificially infested nor was any inoculum added to the soil. An infestation was omitted for the reason that preliminary tests had shown the soil at the station to be already severely infested by *F. culmorum* and further infestation was thought superfluous.

A number of untried commercial-dust seed disinfectants were tested in 1937, 1938, and 1939. For each test diseased wheat was used, but no inoculum was added to the soil. In 1937 and 1938, the wheat belonged to the variety Pentad. This grain was grown in 1935 and originally showed about 90 per cent of the kernels infected by *Helminthosporium sativum.* A part of this lot of wheat had been used in previous experiments, the results of which have already been published (8). In 1939, when the supply of Pentad wheat was exhausted, Mindum wheat, grown in 1938 and with about 40 per cent of the kernels infected with *H. sativum,* was used instead. Supplementary tests with these commercial dusts were made at Treherne in 1937, and at Morden in 1939. In each of these tests the seed was planted in rod rows with a V-belt drill.

In the study of the joint effect of seed disinfection and soil fertilization, 3 seed treatments (New Improved Ceresan, Leytosan, control) and 2 soil treatments (ammonium phosphate, control) were employed in all possible combinations. The seed wheat was planted in 1/100th-acre plots with an ordinary seed drill equipped with a fertilizer attachment. In 1936, 1937, and 1938, clean seed of the variety Renown and, for the 1939 test, diseased seed of the variety Mindum was used. The Mindum wheat was a part of the diseased lot mentioned in the preceding paragraph. The fertilizer was applied at the rate of 54 pounds of the 11–48 mixture per acre, but at Souris, where a supplementary test was made in 1936, the 16–20 mixture was applied at the rate of 88 pounds per acre. In each case the fertilizer was drilled into the soil with the seed.

For the rod-rows experiments just described, the system of replication was 4 to 10 randomized blocks. For the 1/100th-acre plots, 6 × 6 Latin squares were used.

In the rod-row experiments outlined above, the data relative to the influence of the individual seed disinfectants on the emergence of seedlings and the development of root rot in the plants were obtained about a month after the seed was sown. The methods used in obtaining such data were those employed in earlier studies (7). The method of determining the intensity of root rot in the individual plots, however, was based on the revised schedule of root-rot infection classes published by Greaney *et al.* (3).

While, in the rod-row experiments described here, the data on the emergence of seedlings were based on the number of plants emerged from lots of

100 seed each sown in rod rows, in the 1/100th-acre plot experiments, in which the seed was sown with an ordinary drill, such a basis could not be adopted. In these large-plot experiments, therefore, thickness of stand was used as an index of seedling emergence and was determind by pulling and counting all the plants from 8 or 10 1-m. lengths of drill row taken at random from different locations in the same plot. The average count of plants from such locations in any particular plot gave a figure representing the thickness of stand for that plot. To obtain a root-rot rating for the plot, the pulled plants for each of the locations just mentioned were examined individually and classified according to the amount of root rot present. The sum of all readings gave a basis for all disease rating for that particular location, and the average of 8 or 10 such location-ratings gave a figure representing the root-rot rating for the entire plot. The data on thickness of stand and root-rot rating in the 1/100th-acre plots were obtained about 10 days prior to the ripening of the plants.

All data relative to the emergence of seedlings, root-rot ratings, and yield from each experiment were treated statistically by the analysis-of-variance method, and the significance of the observed differences between treatments was determined.

EXPERIMENTAL RESULTS

Comparative Tests with Experimental Organic-mercury Dusts

The 9 untried and unnamed dust seed disinfectants, earlier mentioned, were supplied by F. W. Berk and Co., Ltd., London, England. They were subjected to field tests at Winnipeg in 1935, and at Winnipeg and Souris in

TABLE 1.—*The effect of some experimental organic mercury dusts on emergence of wheat seedlings during 1935 and 1936, the seed being artificially infested with Fusarium culmorum*

Active principle in dust	Percentage of mercury	1935, Winnipeg		1936, Winnipeg		1936, Souris	
		Treated seed	Non-treated seed	Treated seed	Non-treated seed	Treated seed	Non-treated seed
Methyl mercury chloride	1.0	78	76	66	52	98	92
	1.6	84	68	61	45	91	92
	2.2	79	70	68	50	94	96
Methyl mercury nitrate	1.0	84	72	66	51	88	90
	1.6	79	66	66	46	86	90
	2.2	88	82	66	55	94	94
Methyl mercury iodide	1.0	82	84	64	52	86	86
	1.6	86	68	69	52	90	94
	2.2	75	74	70	47	89	97
New Improved Ceresan	3.8	88	76	64	55	97	92
Mean		82.3	73.6	66.0	50.5	90.3	92.3
Standard error of mean		±1.54		±1.15		....	

1936. Three of these dusts contained methyl mercury nitrate, 3 contained methyl mercury chloride, and the remaining 3 contained methyl mercury iodide. Within each group of 3 dusts, the chief difference lay in the percentage of the active principle. Each dust was applied to the seed at the rate of 1 oz. per bu.

These 9 dusts were compared with New Improved Ceresan in respect to their ability to prevent the seedling-blight and root rot of wheat caused by *Fusarium culmorum.* The experimental results (Table 1) showed that, at Winnipeg in 1935 and 1936, the percentage of seedlings emerging from the soil was significantly higher in the plots planted with dusted seed than in those planted with nontreated seed. The differences between treatments in the plots at Souris were found statistically insignificant. The individual dusts had similar effects on the emergence of seedlings, for, in all 3 tests, the observed differences between the dusts were not large enough to be significant. The latter result was surprising, for, owing to differences in chemical composition and in the amount of the active ingredient, some differences between the effects of the dusts were expected.

The effects of the 10 dusts on the root-rot rating in the plots are shown in table 2. In both tests at Winnipeg, the rating was reduced as the result of seed disinfection, but at Souris it was not reduced. Again, in all 3 tests, there seemed to be no difference between the effects of the individual dusts.

TABLE 2.—*The effect of some experimental organic mercury dusts on the root-rot rating in month-old wheat seedlings during 1935 and 1936, the seed being artificially infested with Fusarium culmorum*

Active principle in dust	Percentage of mercury	1935, Winnipeg		1936, Winnipeg		1936, Souris	
		Treated seed	Nontreated seed	Treated seed	Nontreated seed	Treated seed	Nontreated seed
Methyl mercury chloride	1.0	50	50	52	62	20	23
	1.6	44	56	55	67	20	22
	2.2	51	56	52	64	19	17
Methyl mercury nitrate	1.0	44	52	51	63	24	25
	1.6	51	58	49	64	26	23
	2.2	45	50	54	60	18	22
Methyl mercury iodide	1.0	49	51	54	65	31	29
	1.6	46	53	48	64	28	22
	2.2	54	50	49	69	26	19
New Improved Ceresan	3.8	43	50	52	59	17	22
Mean		47.7	52.6	51.6	63.3	23.5	22.4
Standard error of mean		±1.36		±1.01		....	

No definite conclusion could be drawn from the yield data obtained in any of the 3 tests. At Winnipeg, it was found that, in 1935, the plot yields were increased by seed treatment, but, in 1936, no such increase was obtained (Table 3). The plots at Souris were seriously damaged by an invasion of

TABLE 3.—*The effect of some experimental organic mercury dusts on the yield of wheat in 1935 and 1936, the seed being artificially infested with Fusarium culmorum*

Active principle in dust	Percentage of mercury	1935, Winnipeg		1936, Winnipeg	
		Treated seed	Non-treated seed	Treated seed	Non-treated seed
Methyl mercury chloride	1.0	4.4	4.4	16.6	11.8
	1.6	3.6	5.6	15.2	11.6
	2.2	5.8	4.5	18.0	12.4
Methyl mercury nitrate	1.0	4.0	4.0	15.2	11.8
	1.6	5.2	6.8	15.8	11.9
	2.2	4.3	4.2	16.0	12.2
Methyl mercury iodide	1.0	3.0	3.4	14.2	12.3
	1.6	4.3	4.0	17.8	12.0
	2.2	4.8	4.8	17.2	12.0
New Improved Ceresan	3.8	5.1	6.6	16.6	12.8
Mean		4.4	4.8	16.2	12.1
Standard error of mean		....		± 0.44	

grasshoppers and by the drifting of soil from an adjacent plowed field. They were, therefore, considered to be of no value as a source of accurate yield data.

Comparative Tests with Commercial Dusts

Five different dusts were tested in this experiment but no more than 3 in any year. New Improved Ceresan was employed as a standard by which the other dusts were judged. Thus, in 1937, New Improved Ceresan (Canadian Register No. 602), Leytosan (Can. Reg. No. 740), and Half-ounce Leytosan (Can. Reg. No. 942) were tested at Winnipeg and Treherne. In 1938, tests were made at Winnipeg and Morden, the dusts used being New Improved Ceresan, Leytosan, and Half-ounce Leytosan (Can. Reg. No. 989). In 1939, in a test at Winnipeg, the dusts were New Improved Ceresan, Half-ounce Leytosan (Can. Reg. No. 989) and an unnamed dust, supplied by F. W. Berk and Co., Ltd., bearing the symbol IV, 39. Leytosan was applied to the seed at the rate of 1 oz. per bu. of grain; the other dusts were applied at the rate of 0.5 oz. per bu.

The results of these tests are shown in tables 4, 5, and 6. Table 4 shows the effects of the different dusts on the emergence of the seedlings arising from wheat seed infested with *Helminthosporium sativum*. In each of the tests, seed disinfection increased the percentage of seedlings that emerged from the soil, but the dusts seemed to be equally effective. Somewhat similar results (Table 5) were obtained with respect to the root-rot ratings in the plots. In all of the tests, with the exception of the one at Winnipeg in 1939, seed disinfection reduced the root-rot rating. In 1939, owing to unfavorable weather conditions at Winnipeg, root rot in the seedlings did not develop to the usual extent, and, therefore, for this test, no conclusion could be drawn. The effects of the dusts, again, were not strikingly different.

TABLE 4.—*The effect of some commercial organic mercury dusts on the emergence of seedlings from Pentad wheat seed naturally infected with Helminthosporium sativum*

Seed treatment	Winnipeg			Treherne	Morden
	1937	1938	1939	1937	1938
Control	46	49	70	48	52
New Improved Ceresan	79	66	85	84	86
Leytosan	78	66	......	82	85
Half-ounce Leytosan[a]	75	......	......	79	......
Half-ounce Leytosan[b]	......	67	85	......	80
Berk IV, 39	......	......	77	......	......
Standard error	± 1.72	± 2.51	± 1.51	± 2.76	± 2.16

[a] Canadian Register No. 942.
[b] Canadian Register No. 989.

TABLE 5.—*The effect of some commercial organic mercury dusts on the root-rot rating in seedlings from Pentad wheat seed naturally infected with Helminthosporium sativum*

Seed treatment	Winnipeg			Treherne	Morden
	1937	1938	1939	1937	1938
Control	71.2	63.4	16.1	72.7	65.8
New Improved Ceresan	49.1	50.0	13.9	48.6	42.4
Leytosan	50.7	50.7	....	49.6	42.5
Half-ounce Leytosan[a]	51.2	....	....	51.3	....
Half-ounce Leytosan[b]	....	49.6	15.2	....	47.6
Berk IV, 39	....	....	15.1	....	....
Standard error	± 1.32	± 2.02	....	± 1.94	± 1.54

[a] Canadian Register No. 942.
[b] Canadian Register No. 989.

TABLE 6.—*The effect of some commercial organic mercury dusts on the yield of Pentad wheat from seed naturally infected with Helminthosporium sativum*

Seed treatment	Winnipeg			Treherne	Morden
	1937	1938	1939	1937	1938
Control	30.1	18.3	35.9	10.6	32.6
New Improved Ceresan	45.2	22.5	37.6	13.0	42.5
Leytosan	43.1	19.7	....	13.5	43.3
Half-ounce Leytosan[a]	40.1	....	....	14.0	....
Half-ounce Leytosan[b]	....	21.4	32.4	....	41.4
Berk IV, 39	....	....	35.9	....	....
Standard error	± 1.33	± 0.59	....	± 0.75	± 1.66

[a] Canadian Register No. 942.
[b] Canadian Register No. 989.

The results concerning plot yields showed (Table 6) that in 1937 and 1938 the yields were improved by seed disinfection and that there was no marked difference between the abilities of the different dusts to bring about the improvement. In 1939, however, in spite of the fact that seed disinfection improved seedling emergence to a considerable extent, the yield was not improved.

The Joint Effect of Seed Disinfection and Soil Fertilization

As indicated in the review of literature given by Leukel (5), workers with seed disinfectants often observed that treatment of the seed with organic mercury dusts increased the vigor of the subsequent seedlings. In some cases this increase in vigor was attributed to a chemical stimulus received by the seedlings; in other cases it was thought to be the result of the protection afforded the seedlings by the dusts against pathogenic soil-inhabiting microorganisms.

Whatever the reason for this increase in vigor, the present writers were of the opinion that, if seed disinfection was accompanied by soil fertilization, the increase in vigor resulting from the action of the disinfectant might be sustained in later stages of growth by the fertilizer. It was argued that, with soil fertilization, the difference between the yield of strong plants from disinfected seed and the yield of weaker plants from nontreated seed should be considerably greater than if soil fertilization was omitted. Thus, by assuming that seed disinfection and soil fertilization, individually, had beneficial effects on yield, it was considered possible that, if the 2 were employed together, the joint effect would be greater than the sum of the 2 individual effects.

To test the validity of this hypothesis, all possible combinations—6 in all —of 3 seed treatments (New Improved Ceresan, Leytosan, no disinfectant) and 2 soil treatments (ammonium phosphate and no fertilizer) were tested in 1936, 1937, 1938, and 1939. During the first 3 years, disease-free wheat seed was sown, but, in 1939, seed naturally infected with *Helminthosporium sativum* was used instead. The change was made with the object of securing more pronounced differences between treatments. When the field notes were taken, particular attention was given to the effect of the different treatment combinations on plot yields. In addition, data on thickness of stand and root rot were obtained in 1936, 1937, and 1938.

The results from this experiment lent little support to the hypothesis just mentioned. A joint effect of seed disinfection and soil fertilization was indicated in several of the tests, but the results were mostly contrary to what was expected. Thus, in 1936, at Souris, the stand of mature plants was thicker in the fertilized plots sown with disinfected seed than in those receiving other treatments. On the other hand, the results obtained in the fertilized plots at Winnipeg in 1937 showed that seed disinfection reduced the stand. In 2 other tests, the differences produced by the treatments were not significant. The data relative to the root-rot rating in these plots gave very little information as to the merits of the different treatments. In 3 of 4 tests no significant differences between treatments were obtained, and in the 4th test, one made at Winnipeg in 1937, soil fertilization reduced slightly the amount of root rot, while seed disinfection apparently did not.

The most conclusive information relative to the influence of the different treatment combinations were obtained from the data on plot yields. In the 4 tests made in 1936, 1937 and 1938, soil fertilization increased plot yields by significant amounts, but in each test the plots sown with disinfected seed yielded slightly less than those sown with nontreated seed. This effect of the seed disinfection was also observable in the 1939 test, although in this case significant differences between treatments were not established. The tendency for seed disinfection to reduce plot yields frequently occurred in the nonfertilized plots, but the reductions were slightly less marked than in the fertilized plots. The conclusions of Neill (9) that soil fertilization with phosphates offsets the toxicity from organic mercury seed disinfectants were not supported by these results.

The detailed results from this experiment are shown in tables 7, 8, and 9, while a summary of these results is shown in table 10.

TABLE 7.—*The effect of combined seed treatment and soil fertilization on the thickness of stand of wheat plants in 1/100-acre plots at Winnipeg and Souris*

Year and location of test	Soil treatment	Seed treatment			S.E.[a] of seed treatments	Mean of seed treatments	S.E.[a] of mean of treatments
		New Improved Ceresan	Leytosan	Control			
1936, Souris	NP	57	60	49	±2.97	55.3	±1.66
do	Control	50	55	56	±2.97	53.7	
1936, Winnipeg	NP	51	57	53	....	53.7	....
do	Control	38	54	55	....	49.0	
1937, Winnipeg	NP	63	61	68	±1.55	64.0	±1.08
do	Control	64	64	70	±1.55	66.0	
1938, Winnipeg	NP	44	46	44	....	45.5	....
do	Control	46	43	46	....	45.0	

[a] S.E. = Standard error.

TABLE 8.—*The effect of combined seed treatment and soil fertilization on the root-rot rating in wheat plants in 1/100-acre plots at Winnipeg and Souris*

Year and location of test	Soil treatment	Seed treatment			S.E.[a] of seed treatments	Mean of seed treatments	S.E.[a] of mean of treatments
		New Improved Ceresan	Leytosan	Control			
1936, Souris	NP	42	37	40	....	39.7	....
do	Control	40	42	40	....	40.7	
1936, Winnipeg	NP	42	43	43	....	42.7	....
do	Control	45	48	46	....	46.3	
1937, Winnipeg	NP	31	32	31	±1.08	31.3	±0.63
do	Control	39	40	38	±1.08	39.0	
1938, Winnipeg	NP	16	16	16	....	16.0	....
do	Control	17	17	17	....	17.0	

[a] S.E. = Standard error.

It should be remembered that the above results were obtained principally from tests in which disease-free wheat seeds was used. Diseased seed was used in only one test, the one at Winnipeg in 1939, and in this test significant differences between treatments could not be established, chiefly for the rea-

TABLE 9.—*The effect of combined seed treatment and soil fertilization on the yield of wheat in 1/100-acre plots at Winnipeg and Souris*

Year and location of test	Soil treatment	Seed treatment			S.E.[a] of seed treatments	Mean of seed treatments	S.E.[a] of mean of treatments
		New Improved Ceresan	Leytosan	Control			
1936, Souris	NP	9.2	10.2	12.4	±1.08	10.6	±0.64
do	Control	5.2	6.1	5.6	±1.08	5.6	
1936, Winnipeg	NP	15.8	15.5	16.8	±0.53	16.1	±0.33
do	Control	13.0	14.8	15.4	±0.53	14.8	
1937, Winnipeg	NP	29.6	30.0	32.0	±0.70	30.6	±0.41
do	Control	21.6	24.3	22.6	±0.70	22.6	
1938, Winnipeg	NP	21.5	18.6	22.1	±0.83	20.7	±0.52
do	Control	14.6	14.3	15.6	±0.83	14.7	
1939, Winnipeg	NP	25.5	26.6	28.3	....	26.8	....
do	Control	25.9	27.7	26.5	....	26.7	

[a] S.E. = Standard error.

TABLE 10.—*A Summary of the data presented in tables 7, 8, and 9, showing the average effect on thickness of stand, root-rot rating, and yield in wheat grown in 1/100-acre plots at Winnipeg and Souris*

Effect on	Soil treatment	Seed treatment			Mean of soil treatments
		New Improved Ceresan	Leytosan	Control	
Thickness of stand	NP	53.7	56.0	53.5	54.4
	Control	49.5	54.0	56.7	53.4
	Mean	51.6	55.0	55.1	....
Root-rot rating	NP	32.7	32.0	32.5	32.4
	Control	35.2	36.7	35.2	35.7
	Mean	33.9	34.3	33.8	....
Yield	NP	20.3	20.2	22.3	20.9
	Control	16.0	17.4	17.1	16.9
	Mean	18.1	18.8	19.7	....

son that a sufficient amount of disease did not develop. Therefore, although the results obtained in the experiment were contrary to what was expected on the basis of the earlier-mentioned hypothesis, other tests, with diseased seed, seem necessary because, with diseased seed, seed disinfection often increased the yield (Table 6) and by the further use of such seed some support for the hypothesis might be obtained.

The Effect of Organic Mercury Dusts on Disease-free Wheat Seed

It appears (5) that a considerable number of investigators have found seed disinfection to be a decidedly profitable farm practice. It seems, however, that insufficient weight has been given to the fact that this result was obtained with damaged or diseased seed, and the belief that seed disinfection is beneficial under practically all farming conditions has been thus created.

On the contrary, it was shown by Leukel and Stanton (6) that, with oats, the disinfection of clean seed improved crop yields in some localities but not in others, and by Leukel (5), that clean corn seed did not respond to treatment unless sown in cold, wet soil. Koehler (4) found that the disinfection of clean seed of wheat, oats, and barley did not consistently increase the yield, and suggested that seed disinfection was beneficial only when practised on diseased seed. The results obtained by Christensen and Stakman (1) indicated that the benefits derived from seed disinfection were proportional to the amount of disease on the seed.

The results from a number of field experiments in which organic mercurials were used have led the present writers to the conclusion that, under the conditions occurring in Manitoba, the disinfection of clean wheat seed is not a profitable farm practice. In some of these experiments (Table 9 and 10), the treatment of the seed not only failed to increase crop yields but actually decreased them. A review of all the experimental work performed by the writers since 1932 showed (Tables 11, 12, and 13) that, with clean seed, the application of organic mercurials to the seed failed to improve the emergence of seedlings from the soil, or to reduce the amount of root rot in the plants. As already pointed out, the disinfection tended to lower the yield. On the other hand, when the seed was artificially infested with spores of *Fusarium culmorum*, seedling emergence was improved in all cases, the amount of root rot was often reduced, and the yield occasionally increased. With seed naturally infected with *Helminthosporium sativum*,

TABLE 11.—*The effect of seed treatment with organic mercury dusts on the percentage of emerged seedlings from disease-free and diseased seed of wheat*

Year	Number of tests	Condition of seed	Dusted seed	Control	Gain
1932	2	Disease-free	56.7	55.3	+ 1.4
1933	1	do	68.1	72.1	− 4.0
1934	1	do	84.7	81.1	+ 3.6
1935	4	do	87.8	86.5	+ 1.3
1936	4	do	67.3	67.0	+ 0.3
1937	1	do	63.0	69.0	− 6.0
1938	1	do	44.7	45.0	− 0.3
Mean of disease-free seed tests			67.4	68.0	− 0.6
1932	2	Infested with *F. culmorum*	54.2	39.2	+15.0
1933	1	do	63.3	45.1	+18.2
1934	1	do	81.1	74.4	+ 6.7
1935	2	do	80.6	64.9	+15.7
1936	1	do	58.3	31.2	+27.1
Mean of *F. culmorum* tests			67.5	50.9	+16.6
1936	1	Infested with *H. sativum*	86.5	38.0	+48.5
1937	2	do	79.4	47.0	+32.4
1938	2	do	75.1	50.5	+24.6
1939	1	do	82.3	70.0	+12.3
Mean of *H. sativum* tests			80.8	51.3	+29.5

the beneficial effects of disinfection were even more marked. With each kind of seed, there were occasional exceptions to the general rule, but often a reason for the discrepancy could be found.

Concerning the results shown in tables 11, 12, and 13, it may be pointed out that they are the averages of all the tests made in individual years. Where a comparison is made between dusted and nondusted seed, the results for several dusts used in individual tests were averaged and the average was used as a single value to compare with the value obtained from the control. The single values, from the separate tests, for dusted and nondusted seed were then averaged to give the mean for each year. Thus the summarized results are derived from the data obtained from a number of tests made in different ways, in different locations, and in different years. It may be added that, in the course of the tests, some disinfectants were discarded and others used in their place; but, as already shown in this paper, the disinfectants used differed very little in their effect on seedling emergence, root-rot rating, and the yield. It can be assumed, therefore, that the average of the results from several different disinfectants is very similar to the result that would be obtained with any single disinfectant. On this basis, a direct comparison between the result from different years seemed permissible.

TABLE 12.—*The effect of seed treatment with organic mercury dusts on the root-rot rating in wheat plants arising from disease-free and diseased seed*

Year	Number of tests	Condition of seed	Dusted seed	Control	Gain
1932	2	Disease-free	18.7	20.1	+ 1.4
1933	1	do	62.3	57.6	− 4.7
1934	3	do	42.3	45.8	+ 3.5
1935	4	do	46.0	46.4	+ 0.4
1936	4	do	38.2	38.7	+ 0.5
1937	1	do	35.5	34.5	− 1.0
1938	1	do	16.5	16.5	0.0
Mean of disease-free seed tests			37.1	37.1	0.0
1932	2	Infested with *F. culmorum*	19.5	20.5	+ 1.0
1933	1	do	62.3	57.6	− 4.7
1934	1	do	36.7	37.0	+ 0.3
1935	2	do	47.5	57.0	+ 9.5
1936	1	do	58.5	78.1	+19.6
Mean of *F. culmorum* tests			44.9	50.0	+ 5.1
1936	1	Infested with *H. sativum*	50.0	71.2	+21.2
1937	2	do	50.0	71.9	+21.9
1938	2	do	47.1	64.6	+17.5
1939	1	do	14.7	16.1	+ 1.4
Mean of *H. sativum* tests			40.5	55.9	+15.4

The Relation of Seedling Blight and Thickness of Stand to the Yield in Wheat, Oats and Barley

As has already been shown in this paper, the infection of wheat seed by root-rotting fungi results in a seedling blight that reduces the thickness in

TABLE 13.—*The effect of seed treatment with organic mercury dusts upon the yield of wheat from disease-free and diseased seed*

Year	Number of tests	Condition of seed	Dusted seed	Control	Gain
1932	4	Disease-free	23.1	21.5	+ 1.6
1933	3	do	12.8	14.1	− 1.3
1934	3	do	17.7	17.7	0.0
1935	4	do	12.0	12.3	− 0.3
1936	3	do	13.2	13.6	− 0.4
1937	1	do	26.3	27.3	− 1.0
1938	1	do	17.2	18.8	− 1.6
Mean of disease-free seed tests			17.4	17.9	− 0.5
1932	2	Infested with *F. culmorum*	26.2	20.8	+ 5.4
1933	1	do	18.9	14.8	+ 4.1
1934	1	do	32.6	34.3	− 1.7
1935	3	do	6.2	6.3	− 0.1
1936	1	do	15.2	8.4	+ 6.8
Mean of *F. culmorum* tests			19.8	16.9	+ 2.9
1936	1	Infested with *H. sativum*	44.1	30.1	+14.0
1937	2	do	28.1	20.3	+ 7.8
1938	2	do	31.8	25.4	+ 6.4
1939	2	do	30.8	31.6	− 0.8
Mean of *H. sativum* tests			33.7	26.8	+ 6.9

the stand of plants. This reduction in thickness of stand is often accompanied by a reduction in the yield of grain. When the diseased grain is treated with an organic mercury dust, seedling blight is prevented, the thickness of stand improved, and the yield of grain often increased. Occasionally, however, an increase in thickness of stand is secured through seed treatment but there is no increase in the yield of grain, as is shown by the results obtained by the writers in 1934, 1935, and 1939 (Tables 11 and 13.) This departure from the usual is the reason for the experiments discussed here.

At Winnipeg, in 1938 and 1939, disease-free seed of wheat, oats, and barley was sown at 6 different seeding rates, ranging from 50 to 500 seeds to a rod row. The rows were spaced 1 ft. apart and were frequently intertilled to ensure freedom from weeds. Following harvest and threshing, the

TABLE 14.—*The effect of different seeding rates on the yield (grams) of individual wheat, oat, and barley plants at Winnipeg in 1938 and 1939*

Number of seeds per rod row	Wheat		Oats		Barley	
	1938	1939	1938	1939	1938	1939
50	1.620	6.280	3.748	7.414	4.736	5.632
100	1.150	3.940	2.257	4.307	2.840	3.096
200	0.820	2.105	1.273	2.162	1.704	1.900
300	0.586	1.370	0.899	1.622	1.280	1.408
400	0.435	1.207	0.658	1.150	0.984	1.038
500	0.360	0.916	0.554	1.017	0.821	0.805

plot yield and the mean yield of individual plants were determined for each rate of seeding. The data from these experiments are summarized in tables 14 and 15.

TABLE 15.—*The effect of different seeding rates on the yield (bushels per acre) of wheat, oats, and barley at Winnipeg during 1938 and 1939*

Number of seeds per rod row	Wheat		Oats		Barley	
	1938	1939	1938	1939	1938	1939
50	8.1	31.4	33.1	65.5	29.6	35.2
100	11.5	39.4	39.8	76.1	34.5	38.7
200	16.4	42.1	45.0	76.4	42.6	47.5
300	17.6	41.1	47.7	86.0	48.0	52.8
400	17.4	48.3	46.5	81.3	49.2	51.9
500	18.0	45.8	49.0	89.9	51.3	50.3
Standard error	±0.58	±2.31	±1.65	±4.13	±2.74	±3.52
Significant difference	1.63	6.51	4.65	11.64	7.72	9.92

Table 14 shows that the mean yield of individual plants increased as the rate of seeding decreased. This is in accord with the observations made on the growing plants, the thinly-spaced plants appearing to be more vigorous and tillering more freely than the thickly-spaced plants. However, as table 15 shows, with seeding rates of 200 seeds to a rod row and above, the plot yields were at about the same level. Below the 200-seed rate the plot yields were reduced.

Somewhat similar results were obtained from a study of Manitoba wheat fields. Fifty-six fields, well distributed throughout the wheat belt of the Province, were entered and sampled. Ten samples, each sample consisting of all the plants from 1 m. (39.37 in.) of drill row, were collected at random from widely scattered points in each field. The plants obtained from each field were counted and threshed, and from the data thus obtained the mean yield of single plants and the yield of grain per acre for each rate of seeding were calculated. The results of this farm survey are shown in table 16.

It is noteworthy that the trends observed in the experimental plots at

TABLE 16.—*The effect of thickness of stand on the yield of individual plants and on the yield per acre in Manitoba wheat fields in 1939*

Thickness of stand (Number of plants in 16.5 feet of drill row)	Number of fields in class	Average yield of individual plants	Yield per acre
		Grams	*Bushels*
200–250	3	1.307	26.12
251–300	5	0.828	20.88
301–350	17	0.892	26.94
351–400	13	0.696	24.50
401–450	9	0.689	27.68
451–500	6	0.582	26.30
501–550	1	0.561	28.18
551–600	1	0.450	24.86
601–650	1	0.400	24.08

Winnipeg and the trends observed in the farm wheat fields were very similar, although the field methods of cultivation were different. In both cases, the individual plants in a dense stand yielded less than those in a thin stand, and seeding rates of 200 to 500 seeds to a rod row resulted in a more or less uniform level of yields per acre.

It has been pointed out above that the crowded plants in the Winnipeg experimental plots were less vigorous and tillered less than those that were not crowded. In general, the same situation was observed in the farm fields that were sampled. This difference in vigor and tillering of plants seems to account for the difference in their yielding ability, and also for the fact that the yields per acre, over a wide range of seeding rates, persisted at approximately the same level.

It would seem, therefore, that the results obtained from these studies help to provide an answer to the problem introduced in the opening paragraph of this section. It has been stressed already that seedling blight in cereals is associated with an infection or a contamination of the seed with root-rotting fungi. If a loss of these seedlings was the only damage from these root-rotting fungi, it would seem that a great many seedlings could be destroyed without any noticeable effect on the subsequent yield of the crop. The present study shows that up to 60 per cent could be lost in this way without an adverse effect. Conversely, if these seedlings were saved by seed disinfection there also would be little, if any, gain as far as would concern the final yield. Actually, however, the root-rotting fungi carried by cereal seed do not destroy all of the seedlings arising from infected seed. In the majority of cases the seedlings are only weakened and their individual yield is reduced. Consequently, in such cases, seed disinfection not only increases the thickness of stand but also increases vigor of the plants. Thus a gain in yield results, although there is only a small increase in the number of plants that grow. When, as in 1939, for some reason root-rot in the growing plants does not develop, although a number of seedlings are destroyed earlier by blight, the yield need not be reduced unless the loss of seedlings is very great. In other cases, such as occurred at Winnipeg in 1934 and 1935, root rot in the surviving plants does develop; but this damage, combined with the loss of seedlings, is not sufficient to reduce the yield, and, therefore, no beneficial results can be obtained from seed treatment.

DISCUSSION

The salient facts indicated by the experimental results obtained during the present study are: 1. Wheat seed, not infected by root-rotting, smut, or other seed-borne fungi does not respond favorably to treatment with organic mercurials, and 2. With seed infected with root-rotting fungi, the results of the treatment, although often very advantageous, will vary. At the present time, a grower cannot anticipate with any degree of certainty the results he will secure from seed treatment. The treatment is employed chiefly as a precautionary measure, and, as shown in this paper, it may be a costly one. In addition, the experience of the writers indicates that the benefits from seed treatment are likely to vary directly with the amount of disease on the seed and also with the humidity of the climate under which the crop, arising from the treated seed, is grown.

It seems essential, therefore, that, if seed treatment is to be employed to advantage, the health condition of the seed should be determined. The grain grower, usually, is not qualified to make such determinations, as he is improperly equipped to make accurate tests and because the visual examination of the seed, as a criterion of its health condition, is likely to be misleading. Examination of the seed by skilled technicians would permit the grower to learn year by year whether or not seed treatment could be employed with profit. Furthermore, if the seed was found to be free from disease, the likelihood is that he could market his surplus at a premium.

From the results given in this paper, the advantages of sowing disease-free seed are obvious. A yearly seed survey would indicate in what areas seed contamination was most prevalent and in what areas it was least. Such a survey would indicate where seed treatment or the importation of disease-free seed was needed. Preliminary investigations by the writers show that some areas are much more likely than others to produce disease-free seed or relatively disease-free seed year after year. Thus, if further investigations confirm this finding, these areas may be regarded as desirable for seed-grain production. The extent to which grain from such areas could be distributed for seed purposes, however, would, of course, be limited by the cost of transportation.

SUMMARY

In tests of a number of different organic mercury dusts, it was found that these dusts, regardless of differences in chemical composition and in mercury content, had apparently similar effect on the percentage of seedlings that emerged from the soil, on the amount of root rot in the plants, and upon the yield in wheat.

When disease-free seed was disinfected, the treatment tended to decrease the yield, but had no effect on emergence of seedlings or on the amount of root rot. In seed artificially infested with *Fusarium culmorum* or naturally infected with *Helminthosporium sativum,* the emergence of seedlings was always improved by seed disinfection. With but few exceptions, treatment of diseased seed reduced the amount of root rot and increased the yield of grain.

Soil fertilization increased the yield of wheat in field plots sown with disease-free seed. Seed disinfection in combination with the soil fertilization resulted in no further increase in yield.

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LITERATURE CITED

1. CHRISTENSEN, J. J., and E. C. STAKMAN. Relation of *Fusarium* and *Helmintho-*

sporium in barley seed to seedling blight and yield. Phytopath. **25**: 309–327. 1935.

2. GREANEY, F. J., and D. L. BAILEY. Studies in cereal diseases. II. Root-rots and foot-rots of wheat in Manitoba. Dom. of Canada. Dept. Agr. Bull. 85 (n.s.). 1927.
3. ——————, J. E. MACHACEK, and C. L. JOHNSTON. Varietal resistance of wheat and oats to root rot caused by *Fusarium culmorum* and *Helminthosporium sativum*. Sci. Agr. **18**: 500–523. 1938.
4. KOEHLER, B. Seed treatments for the control of certain diseases of wheat, oats, barley. Illinois Agric. Exp. Stat. Bull. 420. 1935.
5. LEUKEL, R. W. The present status of seed treatment, with special reference to cereals. Bot. Rev. **2**: 498–527. 1936.
6. ——————, and T. R. STANTON. Effect of seed treatments on yield of oats. Jour. Amer. Soc. Agron. **26**: 851–857. 1934.
7. MACHACEK, J. E., and F. J. GREANEY. Studies on the control of root-rot diseases of cereals caused by *Fusarium culmorum* (W. G. Sm.) Sacc. and *Helminthosporium sativum* P., K., & B. III. Effect of seed treatment on the control of root rot and the yield of wheat. Sci. Agr. **15**: 607–620. 1935.
8. ——————, and ——————. The "Black-point" or "Kernel Smudge" disease of cereals. Can. Jour. Res. **16** (C): 84–113. 1938.
9. NEILL, J. C. Effect of excess of disinfectant dusts on field germination of wheat. New Zeal. Jour. Agr. **48**: 174. 1934.

PURIFICATION AND PROPERTIES OF ALFALFA-MOSAIC VIRUS PROTEIN

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Since the isolation of tobacco-mosaic virus protein by Stanley in 1935 (16), several other virus nucleoproteins have been obtained in highly purified or crystalline form (3–5, 12, 13, 17, 18). While it is suspected that some of these viruses are transmitted by insects, the specific insect vectors are not known. Potato virus Y and Hyoscyamus virus 3, which are known to be transmitted by insects, were purified by Bawden and Pirie by chemical means (6), but the products were considered impure and largely inactivated. It seemed desirable, therefore, to attempt the isolation in a highly purified and active form of a virus known to be transmitted by a specific insect vector and to compare its properties with those of previously isolated viruses.

Alfalfa-mosaic virus (*Marmor medicaginis* H.)[1] is transmitted by the pea aphid, *Macrosiphum* (*Illinoia*) *pisi* Kalt. (20). The virus can also be transmitted mechanically, and produces local lesions on some varieties of *Phaseolus vulgaris* L., making it possible to obtain quantitative estimations of virus activity. Since the virus is rather unstable, isolation was attempted by means of differential centrifugation at low temperature, a method that has proved well suited for such viruses (18). The results of the application of this method to alfalfa-mosaic virus are here presented, together with a description of some of the properties of the purified material.

EXPERIMENTAL

The virus used is believed to be identical with or a closely related strain of the virus designated by Zaumeyer as alfalfa-mosaic virus 1 (21). Virus activity was determined by the half-leaf method, as described by Loring for tobacco-mosaic virus (11), using *Phascolus vulgaris* var. Early Golden Cluster as the test plant. Unless otherwise stated, all inoculations were made in 0.1 M phosphate buffer at pH 7.1. Plants of Holmes' necrotic-type tobacco (7), derived from Turkish tobacco (*Nicotiana tabacum* L.), were inoculated while young and harvested 10 to 12 days later, since, at greater intervals after inoculation, the plants contain much smaller amounts of virus (14).

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[1] Nomenclature presented in *Handbook of Phytopathogenic Viruses*, by Francis O. Holmes, Burgess Publishing Company (Minneapolis). 1939.

Extraction of Virus

If such plants are ground and pressed without altering the pH of the juice, only a portion of the virus contained in them is removed (14). If sodium hydroxide or dipotassium phosphate is added to the ground plant material in quantities such that the juice will be at about pH 7, the amount of virus obtained is more than doubled. Juice from unfrozen plants is 2 to 4 times as active as that from plants frozen at – 14° C. and nearly twice as active as that from plants frozen rapidly with solid carbon dioxide. The extracted juice (at about pH 7) may be frozen by the latter method without appreciably affecting the activity, while freezing at – 14° C. results in a reduction of 20 to 30 per cent. The losses caused by freezing are probably mechanical in nature, for, as will be shown later, freezing has little or no effect on the virus when purified. Centrifugation on a Swedish angle centrifuge or filtration through celite (Hyflo Super-Cel) does not remove all of the chlorophyll. Filtration through a finer grade of celite (Standard Super-Cel) that removes all chlorophyll also removes about 90 per cent of the virus.

In the method generally used, the harvested plants were cooled to about 4° C., then put through a meat grinder. Three per cent by weight of dipotassium phosphate in the form of a 50 per cent aqueous solution was added to the pulp, which, after thorough mixing, was allowed to stand at 4° C. for about 1 hour. The juice was removed by pressing through bandage gauze, placed in round-bottom flasks or lusteroid centrifuge tubes that were stoppered and placed at – 14° C. or in solid carbon dioxide. When completely frozen, the juice was thawed by rotating the flasks under cold tap water or by centrifuging at room temperature. The juice was then centrifuged on a Swedish angle centrifuge at 4° C. or filtered through celite at 4° C.

Ultracentrifugation

The clarified juice was ultracentrifuged at 0° to 5° C. in a manner similar to that described by Stanley for the isolation of tobacco-ringspot virus (18). About 1½ hours at 30,000 r.p.m. in a field of approximately 60,000 times gravity were required to sediment all of the virus. The pellets were dissolved in 0.1 M or 0.01 M phosphate buffer at pH 7.1, or in one of the foregoing buffers containing 0.5 mg. cysteine per cc., or in such other solvents as will be mentioned later. The insoluble material was removed by low-speed centrifugation at 4° C. for 30 to 60 minutes, and the supernatant liquid containing the virus was again subjected to ultracentrifugation. The process of high- then low-speed centrifugation was repeated 2 or 3 times, but in practically all cases the virus was contaminated by a green material, which contained phosphorus, nitrogen, and carbohydrate. It was found possible to remove this material by centrifuging at 30,000 r.p.m. for 1 minute. The green material was sedimented and the supernatant liquid, containing practically all of the virus, was decanted into clean tubes and ultracentrifuged for 1½ hours. The process of 3 or 4 cycles of alternate high- and low-speed centrifugations, with two short-time high-speed runs resulted in the isolation of a high molecular weight protein, free of the green material and possessing virus activity. The purified material was invariably light-brown, but repeated ultracentrifugation did not appreciably reduce the amount of brown pigment.

Chemical Composition and Yield of Purified Virus

In table 1 are listed data on the yields and analyses of purified preparations obtained by different procedures. The samples used for analysis were dialyzed for 24 hours at 4° C. against distilled water adjusted to pH 7 with

TABLE 1.—*Yields and analyses of ultracentrifugally isolated preparations of alfalfa-mosaic virus*

Nature of starting material	Solvent used	No. of centrifugation cycles	Yield		Phosphorus[a]	Carbohydrate[a]	Carbohydrate: P ratio
			Virus per cc. of juice	Activity			
			mg.	*Per cent*	*Per cent*	*Per cent*	
Unfrozen juice	0.1 M phosphate	3[b]	0.14	30	1.70	10.8	6.4
		3[b]	....	......	1.96	7.9	4.0
		4[b]	....	......	1.77	7.4	4.2
	0.1 M phosphate + cysteine[c]	3	0.19	17	1.38	7.4	5.4
Juice frozen with solid CO_2	0.1 M phosphate	3	0.43	43	1.24	6.5	5.2
		3	0.20	23	1.52	....	......
		3	0.21	20	1.76	11.2	6.4
	0.1 M phosphate + cysteine[c]	3	....	......	1.66	9.3	5.6
		3	0.20	16	1.52	6.6	4.3
Juice frozen at -14° C.	0.1 M phosphate	3[b]	0.09	12	1.85	10.0	5.4
	0.1 M phosphate + cysteine[c]	3	0.32	45	1.32	7.9	6.0
		3	0.17	......	1.35	7.8	5.8
		3[b]	0.14	......	1.73	10.2	5.9
		4[b]	....	......	1.61	8.9	5.5
	0.1 M citrate + cysteine[c]	3	0.21	......	1.29	6.8	5.3

[a] The values in these columns were calculated from analyses for nitrogen, carbohydrate, and phosphorus and are based on the assumption that the nitrogen content of the samples was 16 per cent. Phosphorus was determined by the method of King (9) and carbohydrate with orcinol and sulphuric acid (19).

[b] Isolated from the same batch of plants.

[c] 0.5 mg. per cc.

sodium hydroxide and protected from carbon dioxide with a soda lime tube. This procedure was necessary to prevent the isoelectric precipitation of the virus by dissolved carbon dioxide. Dialysis under these conditions does not appreciably affect virus activity. In 7 tests, the dialyzed preparations averaged 89 per cent as active as controls kept at 4° C. The phosphorus and carbohydrate contents of the several preparations varied considerably, the averages being 1.58 per cent and 8.5 per cent, respectively. The true phosphorus content is probably more nearly 1.3 per cent, a value approached by several of the samples. Those samples that contained the larger amounts of phosphorus were possibly incompletely dialyzed or insufficiently purified. The latter possibility is suggested by the tendency of the 4-times-centrifuged samples to be lower in both phosphorus and carbohydrate than the corresponding samples that were centrifuged only 3 times. On the assumption that the phosphorus and carbohydrate are present as nucleic acid, the carbohydrate: phosphorus ratio was high in nearly all samples. The excess carbohydrate was probably an impurity and was quite possibly associated with the brown pigment. A highly active preparation was well dialyzed against water, then frozen and dried. It contained 53.87 per cent carbon, 6.69 per cent hydrogen, 16.25 per cent nitrogen, 1.44 per cent phosphorus, and 0.65 per cent sulphur. The analyses are those that would be expected for a nucleoprotein containing about 14–16 per cent nucleic acid and a few per cent extraneous carbohydrate.

Apparently, some loss of activity occurred during the purification process, for the activity of the final product usually accounted for but 20 to 40 per cent of that of the starting material (Table 1, column 5). These values were obtained by direct comparison of the activity of a 1:10 dilution of the juice with that of known dilutions of the purified material. Since the volumes of the juice and of the purified preparation were recorded, the percentage recovery could be calculated. It appears likely that much of the loss in activity was due to actual loss of virus and not to inactivation, for during the procedure comparatively large amounts of insoluble precipitates, known to have absorbed appreciable quantities of virus, were discarded. It is possible, however, that some inactivation did occur and that the material isolated contained partially inactivated virus.

The results presented in table 1 indicate that neither the method of preparing the juice nor the solvent used for dissolving the pellets had much effect on either the yield or the composition of the purified protein. There was, however, an effect on other properties. The use of cysteine generally resulted in preparations that possessed more activity and a higher degree of homogeneity when examined in the ultracentrifuge. The green contaminant was most easily removed when the juice was frozen at − 14° C. In other experiments very poor yields of protein, badly contaminated with the green material, were obtained when the juice was not frozen. When distilled water containing cysteine and adjusted to pH 7 was used, the isolated protein was mostly inactive and quite heterogeneous, while the use of distilled water alone resulted in very poor yields. The use of citrate buffer resulted in a preparation about 70 per cent as active as one prepared from the same material with phosphate buffer.

It may be calculated from the data in the 4th and 5th columns of table 1 that the expressed juice of diseased plants contains about 1 mg. of virus per cc. A similar value also is indicated by the fact that juice diluted 1:10 produces about the same number of lesions on bean leaves as does the purified material at a concentration of 10^{-4} gm. per cc. This amount is present only during the winter months, as indicated by the fact that much smaller yields are obtained during the summer. During the latter season it is also much more difficult to remove the green contaminant, and the purified samples are high in phosphorus and carbohydrate.

Preliminary Concentration of the Virus

Attempts were made to effect a preliminary concentration of the virus, so that the amount of ultracentrifuging could be reduced. Since it was found that a single precipitation with 30 per cent ammonium sulphate in the cold had little if any effect on the activity of the virus, this salt was used to precipitate the virus from the juice. The precipitated virus was dissolved in 0.1 M phosphate buffer containing 0.5 mg. cysteine per cc. and equal in volume to $\frac{1}{10}$ that of the original juice. This was then subjected to 3 centrifugation cycles in the usual manner. In a control experiment, virus was isolated from some of the same juice by the usual 3 centrifugation cycles, using 0.1 M phosphate buffer containing cysteine as solvent for the pellets. The hydrogen-ion concentration of still another portion was adjusted to pH 4.6 with acetic acid. The precipitate was removed by centrifugation and treated exactly the same as the ammonium-sulphate-precipitated virus. All operations were carried out at 4° C. The preparations obtained were compared with respect to activity, then dialyzed at 4° C. for 24 hours, analyzed, and again compared for activity. As may be seen from the results given in table 2, both methods of concentration resulted in smaller yields and less active preparations, although that treated with ammonium sulphate was nearly as active as that isolated by ultracentrifugation alone. The sample precipitated with acetic acid was much lighter in color than any other preparation so far obtained, while the one precipitated with ammonium sulphate was much darker and less homogeneous, as indicated by the fact that continued low-speed centrifugation continued to remove material. The significance of the differences in composition is lessened by the unaccountably high phosphorus content of the control. Because of the effect upon activity, these methods were not used.

It was found possible to obtain some concentration by simply freezing the juice in lusteroid tubes, then centrifuging at room temperature until thawed, but no longer, and siphoning off the upper $\frac{2}{3}$ of the supernatant liquid. The virus, together with most of the pigment, was found to be in the lower third. This method of obtaining approximately a threefold con-

TABLE 2.—*Yields, relative activities, and analyses of purified virus preparations obtained by ultracentrifugation following preliminary concentration*

Method of concentration	No. of centrifugation cycles	Yield per cc. of juice	Relative activity[a]		Phosphorus[b, c]	Carbohydrate[b]
			Before dialysis	After dialysis[c]		
		mg.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
$(NH_4)_2SO_4$ 30 per cent	3	0.14	68	63	1.82	6.8
Acetic acid pH 4.6	3	0.10	41	36	1.34	5.6
Ultracentrifugation alone	3	0.24	100	88	2.06	7.6

[a] The figures in these columns were obtained by dividing the number of lesions produced when the designated sample was rubbed on 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster by the number produced when a solution of the control of the same concentration was rubbed on the opposite halves, and multiplying by 100. The control used in each case was the solution of virus isolated entirely by ultracentrifugation and stored at 4° C.

[b] The values in these columns were calculated from analyses for nitrogen, carbohydrate, and phosphorus and are based on the assumption that the nitrogen content of the sample is 16 per cent. Phosphorus was determined by the method of King (9) and carbohydrate with orcinol and sulphuric acid (19).

[c] The samples upon which these analyses and activity measurements were made were dialyzed in a rocking dialyzer at 4° C. for 24 hours against 50 liters of distilled water at about pH 7.

centration of virus has been used rather extensively. The success of the method is probably due to the fact that the pure ice crystals, which concentrate at the top, are the last to thaw.

General Properties

Solutions of the virus conatining 5 to 10 mg. per cc. are only slightly opalescent and do not exhibit stream double refraction. The jelly-like pellets obtained by ultracentrifugation are only slightly birefringent. This may be due to the photoelastic effect, for it is increased by creating a strain in the pellet by pressing with a needle. Unlike tobacco-mosaic virus, samples of alfalfa-mosaic virus, when brought to the isoelectric point, do not form crystals nor is there any indication of a sheen. It appears likely, therefore, that the particles are essentially spherical in shape. Ultracentrifugation studies indicated that the virus has a sedimentation constant of approximately 74×10^{-13} cm./sec. in unit centrifugal field (10). The preparations did not possess the same degree of homogeneity with respect to rate of sedimentation or electrophoretic mobility as tobacco-mosaic virus. The results indicated that each preparation consisted of a continuous distribution of particle sizes with a limited range about a modal value. The density of the protein, measured by means of a pycnometer, was found to be 1.48. Assuming a spherical shape, alfalfa-mosaic virus protein therefore has a molecular weight of about 2.1×10^6 and a diameter of 16.5 mμ. The isoelectric point estimated from the point of minimum solubility was pH 4.6 (10).

In the absence of salt, the protein is soluble in 90 per cent alcohol and to a limited extent in hot 5 per cent trichloroacetic acid. However, if salt is present, the virus is insoluble in both solvents. Attempts to crystallize the virus by the use of ammonium sulphate or by dialysis were unsuccessful.

All of the more carefully prepared samples had about the same specific activity, *i.e.*, a concentration of 10^{-4} g. per cc. caused 20 to 40 lesions per half leaf and one of 10^{-5} g. per cc. an average of about 1 lesions. Several of the preparations were rubbed on leaves of *Nicotiana glutinosa* L. and found to be free of tobacco mosaic virus. When the purified protein was rubbed on tobacco leaves, typical symptoms of alfalfa mosaic were produced.

Isolation of Nucleic Acid

Since alfalfa-mosaic virus appeared to be a nucleoprotein, an attempt was made to isolate nucleic acid from it by the method of Johnson and Harkins (8) for the isolation of yeast nucleic acid. In one experiment, sufficient concentrated sodium hydroxide to make a 5 per cent solution was added to 4 cc. of a solution at 0° C. containing 40 mg. of protein. After standing at 0° for 1 minute, the solution was made acid to litmus by the addition of glacial acetic acid and the precipitated protein removed by centrifugation and then washed once with a small volume of water. The supernatant liquid plus the wash water was acidified to Congo red with concentrated hydrochloric acid and then treated with an equal volume of alcohol. The precipitated nucleic acid was removed by centrifugation and, after being washed 3 times with alcohol and dried *in vacuo* over phosphorus pentoxide at 110° C., weighed 3.1 mg. The material was dissolved in dilute sodium hydroxide and, upon analysis, found to contain 14.2 per cent nitrogen by the Kjeldahl method, 9.8 per cent phosphorus by the method of King (9), and 41 per cent carbohydrate by the orcinol method (19). The isolation experiment was repeated, using 161.5 mg. protein in 15 cc., and 14.5 mg. nucleic acid were obtained. Upon combustion, the latter was found to contain 34.47 per cent carbon, 3.73 per cent hydrogen, 8.34 per cent phosphorus, and 15.41 per cent nitrogen. The material is similar to yeast nucleic acid in elementary composition and, like it, gives negative Dische and Feulgen reactions. It may be concluded that alfalfa-mosaic virus is a nucleoprotein containing a nucleic acid of the type obtained from yeast and several other viruses. Only about 60 per cent of the phosphorus present in the protein was isolated in the form of nucleic acid, but, since viruses differ in the ease with which nucleic acid is split off with sodium hydroxide (18), it is possible that the hydrolysis may have been incomplete. The fact that the protein component obtained in the second isolation experiment was found to contain 0.2 per cent phosphorus and 2 per cent carbohydrate is in accordance with this viewpoint.

Dilution Curve

The dilution curves of a number of preparations in different solvents were studied so that optimum conditions for activity measurements could be determined. Several different concentrations of virus protein were prepared in a given solvent and their activities compared with that of a control containing 10^{-4} g. per cc. in the same solvent. The dilution curves of a typical preparation in 0.1 M and 0.01 M phosphate buffers are given in figure 1. The results are expressed as relative number of lesions given by each dilution, taking the number given by the control as 100 per cent. In 0.1 M phosphate buffer, the number of lesions is proportional to the virus concentration within a limited range. At greater concentrations, as is the case with many viruses, the dilution curve has a slope of less than unity. However, at dilutions higher than 5×10^{-4} g. per cc., the slope becomes much greater than unity. This last effect has been observed also with juice containing alfalfa-mosaic virus (14) and with certain other viruses (2). The dilution curves of several samples of alfalfa-mosaic virus protein were determined and all were of the same general shape. In 0.01 M phosphate buffer the dilution curve has a slope of less than one, except at very high dilutions. Such a solvent was considered unsuited for activity measurements; hence, 0.1 M phosphate buffer was used in nearly all cases. An

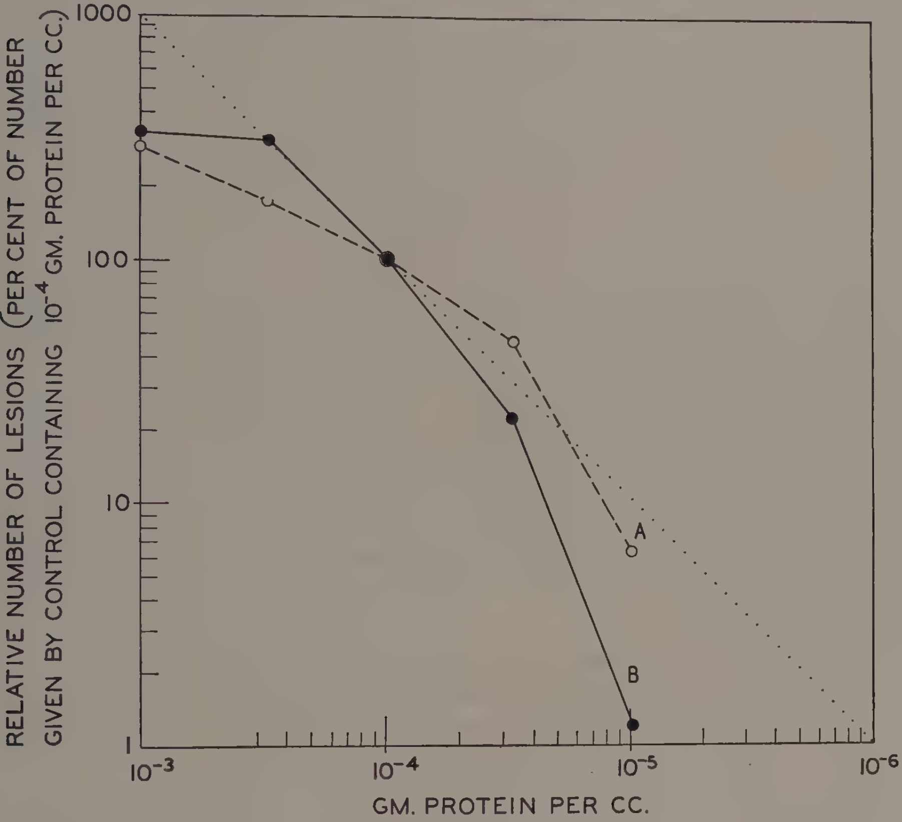

FIG. 1. Dilution curve of purified alfalfa-mosaic virus protein. Curves A and B are the dilution curves of the sample in 0.01 M and 0.1 M phosphate buffers at pH 7.1, respectively. The dotted line represents a theoretical dilution curve of slope 1.

attempt was always made to inoculate at a concentration not far from 10^{-4} g. per cc., for in that region the number of lesions is approximately proportional to the virus concentration. The accuracy to be expected when these precautions are taken is indicated by the data in table 3. Samples differing in protein concentration by 20 per cent were compared with a common control containing 10^{-4} g. per cc. by the method previously described. In 4 of the tests the concentrations estimated from activity data did not differ by more than 10 per cent from the actual concentration, and in the other 2 tests the difference was less than 20 per cent.

TABLE 3.—*Accuracy of activity measurements*

Virus concentration	Relative virus concentration	Relative number of lesions[a]
g./cc.	*Per cent*	*Per cent*
1.4×10^{-4}	140	124.0
1.2×10^{-4}	120	117.5
1.0×10^{-4}	100	83.2
0.8×10^{-4}	80	71.0
0.6×10^{-4}	60	63.8
0.4×10^{-4}	40	34.8

[a] The figures in this column were obtained by dividing the number of lesions produced when the designated sample was rubbed on 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster by the number produced when a solution containing 10^{-4} g. of virus per cc. was rubbed on the opposite half leaves, and multiplying by 100. All inoculations were made in 0.1 M phosphate at pH 7.1.

The total number of lesions produced by a given sample is also influenced by the solvent (Table 4). Hence, when the relative activity of 2 or more samples was estimated, care was taken that they were in the same solvents.

The specific activity of purified preparations of alfalfa-mosaic virus appears to be considerably less than that of other viruses isolated in this laboratory. This is not sufficient reason for believing, however, that the virus

TABLE 4.—*Effect of solvent on number of lesions produced by purified alfalfa-mosaic virus*

The figures represent the numbers of lesions per half leaf produced on 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster by virus preparations of the designated concentration dissolved in the designated solvent. The samples paired vertically were rubbed on opposite half leaves. All solutions were at pH 7.1.

Solvent	Virus concentration	
	10^{-4} g./cc.	10^{-5} g./cc.
0.1 M phosphate	58.5	0.3
0.01 M phosphate	102.9	4.7
0.1 M phosphate	25.5	0.5
0.01 M phosphate	30.9	3.2
0.1 M phosphate	13.7	......
0.1 M citrate	4.1	......
0.01 M phosphate	17.6	......
0.01 M citrate	6.5	......

was inactivated during the isolation procedure, as was thought to be the case with potato virus Y and Hyoscyamus virus 3 (6). If any inactivation occurred during the purification of alfalfa-mosaic virus, it could not have been great, for in some cases approximately half of the total activity represented by the starting material was recovered in the isolated nucleoprotein; hence, the loss was no greater than might be expected to occur due to mechanical reasons. It is possible that the specific activity may appear low owing to the host used for activity determinations, for the amount of material required to cause infection when rubbed on plants varies considerably with the species and with the variety of the plant.

pH Stability

To aid in the selection of the optimum conditions for isolation and for storage, the stability of the virus at different hydrogen-ion concentrations was determined. Five-cc. portions of a well-dialyzed virus solution containing 2×10^{-3} g. per cc. were cooled to 4° C. and added to equal quantities of cold 0.075 M phthalate-phosphate-borate buffer previously adjusted to different hydrogen-ion concentrations with sodium hydroxide or hydrochloric acid. The hydrogen-ion concentrations were then determined at room temperature and the samples were stored at 4° C. Immediately after mixing

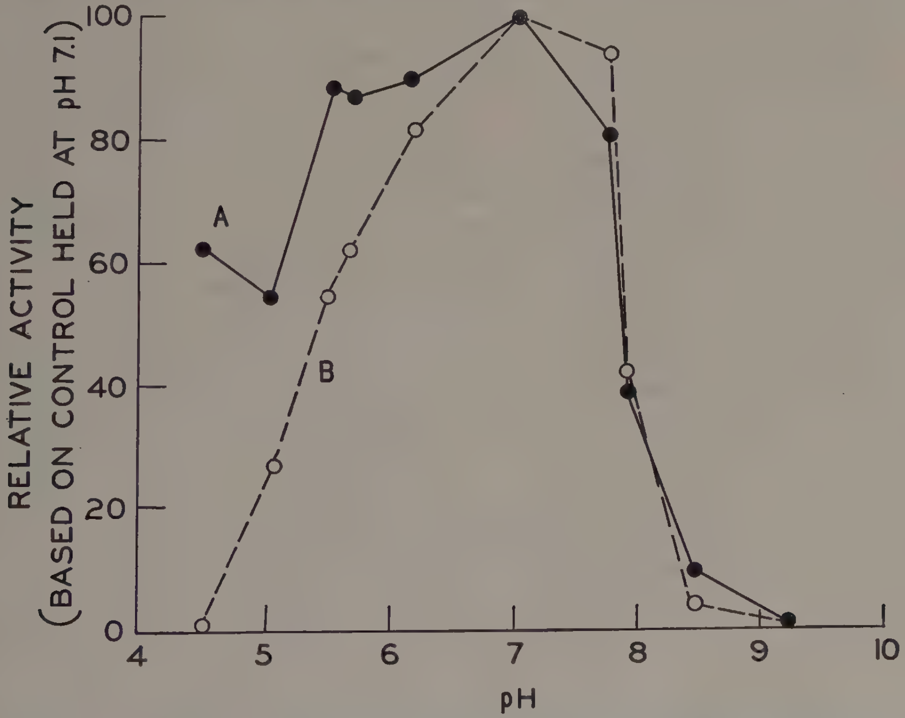

FIG. 2. Stability of purified alfalfa-mosaic virus at 4° C. at different hydrogen-ion concentrations. Curves A and B represent the relative activity of the samples after standing at the various pH values for 20 hours and 120 hours, respectively. They were adjusted to pH 7.1 immediately before being used for inoculation.

and at intervals samples were removed, diluted 1:10 with 0.1 M phosphate buffer at pH 7.1, and used for activity measurements. One of the samples, that at pH 7.1, was taken as the control and each sample was compared with it. The hydrogen-ion concentration of the solutions used for inoculation did not differ by more than 0.1 pH unit. The results are presented in figure 2. The virus is most stable at about pH 7 and is fairly stable over the range pH 5.5 to 7.7. This is a narrower pH stability range than has been reported for other plant viruses. The virus is much more stable at pH 7 than it is at its isoelectric point, pH 4.6. The experiment was repeated twice, once with purified virus and once with partly purified virus. Comparable results were obtained.

Effect of Storage

In figure 2, the results are presented as relative changes in activity, and, consequently, give no indication of the stability of the control. Since the actual number of lesions produced by a sample is affected by many environmental factors, the average number of lesions produced by a sample on succeeding days is not a good measure of changes in the activity of the sample. If the virus is not affected by freezing and thawing, changes in the activity of a sample could be followed by comparing with a control kept in the frozen state. It appeared desirable, therefore, to determine the effect of freezing and thawing on the virus under different conditions.

Samples containing 1 mg. of alfalfa-mosaic virus per cc. in nutrient broth, in 0.1 M phosphate buffer, and in water were frozen rapidly with carbon dioxide and left frozen for 20 hours. They were then thawed, diluted with 0.1 M phosphate, and compared for activity with similar samples stored at 4° C. The samples that had been frozen were 80, 86, and 85 per cent as active, respectively, as the unfrozen controls. Corresponding samples placed in a room held at – 14° C. for the same length of time were 79, 89, and 69 per cent as active, respectively, as the controls. The experiment was repeated and similar results were obtained. In further tests, samples in 0.1 M phosphate buffer that were frozen for short periods of time in carbon dioxide averaged 94 per cent as active as the controls. While most of these differences are not significant when considered individually, the fact that they are all in the same direction indicates that a small amount of inactivation may result from freezing and thawing. Alfalfa-mosaic virus, therefore, differs from tobacco-ringspot virus (18), which is denatured by freezing in water. When solutions of alfalfa-mosaic virus are frozen and thawed, there is no indication of denaturation, for no insoluble precipitate is formed. However, unless samples are sealed in glass, those frozen by means of carbon dioxide and containing no buffer may absorb sufficient carbon dioxide to cause the isoelectric precipitation of the protein.

A solution of purified virus containing 1 mg. per cc. in 0.1 M phosphate at pH 7.1 was prepared, and, since freezing and thawing have only a slight effect on the virus, aliquot portions to be used as controls were sealed in glass and stored in solid carbon dioxide. Others were stoppered and stored at 4° C., and, since, even at that temperature, some bacterial action occurs, toluene was added to some. At intervals, samples were removed and used for activity comparisons. In one experiment there were no significant differences in the activity of the several lots up to a period of 4 days. In another experiment there were no differences apparent in 3 days; but, in 12 days, the samples stored without toluene at 4° C. were 44 per cent as active as the control stored in the frozen state, while those to which toluene was added were 65 per cent as active as the controls. After a period of 27 days the percentages were 44 and 62 without and with toluene, respectively. The data indicate that the purified virus is relatively stable at 4° C. in 0.1 M phosphate buffer at pH 7.1, but that inactivation does occur over a long period of time. The addition of toluene has a slight effect in preserving virus activity. It is possible that the activity of the frozen samples decreases with time, but this does not appear probable, for the samples kept frozen for 27 days still produced about as many lesions per half leaf as they did when the experiment was started, indicating that no great change in activity had occurred. Although the virus is fairly stable at 4° C., inactivation proceeds rather rapidly at higher temperatures. A sample in 0.1 M phosphate buffer and containing toluene was held at 24° C. and lost 72 per cent of its activity in 1 day, 74 per cent in 2 days, and 95 per cent in 4 days.

A comparison was made of the stability of the virus in distilled water, and in 0.01 M and 0.1 M phosphate buffers at pH 7. After 6 days at 4° C., the sample in 0.1 M phosphate buffer was 59 per cent as active as that in 0.01 M phosphate buffer and about twice as active as that stored in water. After 28 days, the sample in 0.1 M phosphate was only 19 per cent as active as that in 0.01 M phosphate and 52 per cent as active as that in water. Repetition of the experiment gave comparable results. Hence, the virus is more stable in 0.01 M phosphate buffer than it is in 0.1 M phosphate. The behavior in the absence of buffer is somewhat erratic, probably because of changes in pH brought about by absorption of carbon dioxide. Activity is lost much more rapidly in juice than in purified solutions. A sample of juice at pH 7 stored at 4° C. lost 52 per cent of its activity in 3 days and 87 per cent in 7 days, when compared to a portion of the same juice stored in the frozen state.

Effect of Reducing Substances on the Virus

Since cysteine and other reducing agents have a stabilizing effect on some plant viruses (1, 18), it appeared desirable to determine their effect on alfalfa-mosaic virus. In one experiment conducted at 4° C., pellets obtained by ultracentrifugation of infectious juice were dissolved in 0.1 M phosphate buffer at pH 7.1 equal in volume to $\frac{1}{4}$ that of the original juice. In a second experiment conducted at 24° C., a solution of purified virus containing 1 mg. per cc. was used. Solutions of cysteine, sodium sulphite, and sodium hydrosulphite were adjusted to pH 7 with sodium hydroxide and then added to different portions of the virus solution. At intervals, samples were diluted 10 times with 0.1 M phosphate buffer and compared for activity with controls treated in exactly the same manner, except that water was added instead of the solutions of reducing agents. It may be seen from table 5 that cysteine has no effect on purified virus but that it tends to stabilize partially purified virus. Because of these results, cysteine was usually used in the purification process. On the other hand, sulphite and hydrosulphite inactivate the virus. The cysteine probably protects the virus from the inactivating effect of some substance that is eliminated by the purification process.

TABLE 5.—*Effect of reducing substances on alfalfa-mosaic virus*

[The figures represent the number of lesions per half leaf produced when 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster were rubbed with a 1: 10 dilution of the indicated sample.]

Type of preparation	Hours	Temperature	Cysteine[a]		Sodium sulphite[a]		Sodium hydrosulphite[a]	
			Sample	Control	Sample	Control	Sample	Control
		°C.						
Ultracentrifuged 1 time	0	4	45.8	35.8	26.7	28.8	14.2	37.4
	14	4	15.7	15.3	15.8	14.6	14.2	17.4
	60	4	9.6	8.4	1.4	10.9	2.8	10.0
	120	4	24.3	13.3	0.5	10.8	1.0	13.4
Ultracentrifuged 4 times	0	24	13.4	22.7	13.8	8.7	......	....
	24	24	1.9	2.6	0.2	5.3	......	....
	48	24	1.1	2.4	0.1	4.0	......	....
	96	24	0.1	0.3	0.2	1.1	......	....

[a] 1 mg. per cc.

Effect of Trypsin

Since trypsin has been successfully employed in the purification of tobacco-mosaic virus and its strains (3, 15), the possibility of using it to remove the brown coloring matter associated with alfalfa-mosaic virus was investigated. Crystalline trypsin, kindly supplied by Dr. M. Kunitz, was added to a solution of alfalfa-mosaic virus in 0.1 M phosphate buffer at pH 7.1 at a ratio of 1: 100 and allowed to stand at room temperature. At intervals, samples were removed, diluted to a virus concentration of 10^{-4} g. per cc. with 0.1 M phosphate buffer, and compared for activity with a control treated in exactly the same manner, except that no trypsin was added. The results shown in table 6 indicate that the enzyme inactivates the virus, although there seems to be an initial activating effect. The inactivation is not merely an inhibition of the virus, as is the case with that of tobacco mosaic, for there is no immediate inhibition, and the inactivation is a function of time and proceeds at a measurable rate.

In order to establish more definitely the nature of the action of trypsin on alfalfa-mosaic virus, an experiment was conducted in which the rate of hydrolysis of heavy protein was also followed. To 3-cc. portions of alfalfa-

TABLE 6.—*Rate of inactivation of alfalfa-mosaic virus by trypsin*

[The reaction mixtures consisted of 4.3 mg. virus and 0.043 mg. trypsin per cc. in 0.1 M phosphate buffer at pH 7.1.]

Temperature	Time	Lesions		Relative activity of trypsin-treated sample
		Trypsin-treated[a]	Control[a]	
	hr.			*Per cent*
25° C.	0	68.4	46.7	147
	1	23.0	27.2	85
	3	5.8	14.9	39
19° C.	0	58.1	41.3	141
	1	32.6	37.1	88
	3	1.7	14.5	11
	6	3.3	45.8	7
	24	1.1	8.3	13

[a] The figures in these columns represent the number of lesions per half leaf produced when the designated sample and the corresponding control were rubbed on opposite half leaves of 24 or more leaves of *Phaseolus vulgaris* var. Early Golden Cluster. The samples were diluted 1: 10 with 0.1 M phosphate buffer at pH 7.1.

mosaic virus containing 4.82 mg. protein per cc. in 0.1 M phosphate buffer at pH 7.1, 0.7-mg. portions of crystallized trypsin were added. Immediately after mixing and at intervals up to 4 hours, individual samples were diluted to 15 cc. with cold water to retard the reaction and ultracentrifuged at 0–5° C. for 1½ hrs. at 30,000 r.p.m. to separate the virus and enzyme. The upper half of the supernatant liquid was drawn off and analyzed for total and for protein nitrogen. The pellets were redissolved in 15 cc. of cold phosphate buffer at pH 7 and the virus ultracentrifuged again. The second pellets were dissolved in a small volume of buffer and centrifuged at low speed. The supernatant liquids were then analyzed for nitrogen and their activities compared with that of a control treated in exactly the same manner, except that no trypsin was added. The samples were diluted to 10^{-4} g. per cc., assuming that all of the nitrogen present was protein nitrogen. The results are presented in table 7. It is evident that hydrolysis occurred, for the amount of heavy protein recovered decreased as the time of contact with the enzyme was lengthened and most of the nitrogen was recovered in the supernatant liquid. The rate of hydrolysis, as well as that of inactivation, was not so great at 4° C. as at the higher temperature. The nitrogen not sedimented in the ultracentrifuge was approximately equally divided between low molecular weight protein and non-protein nitrogen. As the reaction proceeded, there was a decrease not only in the amount of heavy protein recovered, but also in the activity of the material isolated. After 2 to 4 hours, the heavy protein that remained intact had lost about 99 per cent of its activity. The obvious conclusion is that inactivation proceeds at a faster rate than does the extensive cleavage of the heavy protein. The possibility exists, however, that the material isolated was not entirely protein. Nucleic acid might possibly be split off in fragments sufficiently large to be sedimented by the ultracentrifugation. The quantity isolated was too

TABLE 7.—*The effect of trypsin on alfalfa-mosaic virus*

[Each sample consisted of 14.4 mg. virus, corresponding to 2.31 mg. N, and 0.7 mg. trypsin dissolved in 3 cc. of 0.1 M phosphate buffer at pH 7.1].

Time	Temperature	Supernatant liquid from first ultracentrifugation		N recovered as heavy protein	Relative activity of recovered protein[a]
		Total N	Protein N[b]		
Minutes	*°C.*	*Mg.*	*Mg.*	*Mg.*	*Per cent*
Control	4	0.36	0.27	1.05	100
0	24	1.11	0.58	0.75	85
30	24	1.57	0.90	0.52	35
60	24	1.72	1.02	0.42	10
120	24	1.87	0.96	0.34	1
240	24	2.00	0.99	0.24	1
240	4	1.41	0.75	0.66	53

[a] The figures in this column were obtained by dividing the number of lesions produced when a solution of the designated sample containing 10^{-4} g. protein per cc. was rubbed on 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster by the number produced when a solution of the control of the same protein concentration was rubbed on the opposite halves, and multiplying by 100.

[b] Insoluble in 15 per cent trichloroacetic acid.

small to permit examination of its properties in detail, but it resembled the virus protein in appearance. If trypsin actually has an initial activating effect, as suggested by the data in table 6, it must be due to the presence of the enzyme and not to any permanent effect on the virus, for the activity of virus exposed to trypsin for a brief time and then separated from the enzyme did not differ significantly from that of the control.

SUMMARY

A high molecular weight nucleoprotein possessing the properties of alfalfa-mosaic virus, a virus known to be transmitted by a specific insect vector, has been isolated from diseased tobacco plants by means of differential centrifugation at a temperature of 0° to 4° C. The virus preparations contained about 15 per cent of a nucleic acid of the yeast nucleic acid type, about 0.65 per cent sulphur, and small amounts of extraneous carbohydrate. The virus appears to be essentially spherical in shape and has a specific gravity of 1.48, and a sedimentation constant of approximately 74×10^{-13}. It is the smallest plant virus that has been isolated, for the molecular weight and diameter, based on the foregoing constants, are 2.1×10^{6} and 16.5 mμ, respectively.

Freezing and thawing have only a slight effect on the purified virus, but similar treatment of diseased plant material results in a measurable loss of virus. The virus, when purified, is reasonably stable at 4° C., but is rapidly inactivated at room temperature and is much less stable when in juice. Sodium sulphite and sodium hydrosulphite inactivate the virus, while cysteine has a stabilizing effect on partly purified preparations, but no effect on purified ones. The virus is slightly more stable in 0.01 M phosphate buffer than in 0.1 M phosphate buffer or in water. Toluene helps to preserve the virus.

The juice of diseased greenhouse-grown tobacco plants contains about 1 mg. of virus per cc. during the winter months and less in summer. When virus dissolved in 0.1 M phosphate buffer at pH 7 is rubbed on bean leaves, the number of lesions produced is proportional to the concentration of virus over a limited range only. At concentrations less than 5×10^{-4} g. per cc., the number produced decreases more rapidly than does the virus concentration in the inoculum, and, at 10^{-5} g. per cc., few or no lesions result. The virus is inactivated and hydrolyzed by trypsin. The inactivation proceeds at a more rapid rate than does the extensive hydrolysis of the high molecular weight protein. In so far as has been determined, alfalfa-mosaic virus nucleoprotein does not differ fundamentally from other virus nucleoproteins that have been isolated and for which no specific insect vectors are known.

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LITERATURE CITED

1. BALD, J. G., and G. SAMUEL. Some factors affecting the inactivation rate of the virus of tomato spotted wilt. Ann. Appl. Biol. **21**: 179–190. 1934.
2. ————. The use of numbers of infections for comparing the concentration of plant virus suspensions. II. Distortion of the dilution series. Ann. Appl. Biol. **24**: 56–76. 1937.
3. BAWDEN, F. C., and N. W. PIRIE. The isolation and some properties of liquid crystalline substances from solanaceous plants infected with three strains of tobacco mosaic virus. Proc. Roy. Soc., Ser. B. **123**: 274–320. 1937.
4. ————, and ————. Liquid crystalline preparations of potato virus "X." Brit. Jour. Exp. Path. **19**: 66–82. 1938.
5. ————, and ————. Crystalline preparations of tomato bushy stunt virus. Brit. Jour. Exp. Path. **19**: 251–263. 1938.
6. ————, and ————. The purification of insect-transmitted plant viruses. Brit. Jour. Exp. Path. **20**: 322–329. 1939.
7. HOLMES, F. O. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopath. **28**: 553–561. 1938.
8. JOHNSON, T. B., and H. H. HARKINS. Researches on pyrimidines. CVII. The examination of yeast nucleic acid for 5-methylcytosine. Jour. Am. Chem. Soc. **51**: 1779–1784. 1929.
9. KING, E. J. The colorimetric determination of phosphorus. Biochem. Jour. **26**: 292–297. 1932.
10. LAUFFER, M. A., and A. F. ROSS. Physical properties of alfalfa mosaic virus. Jour. Am. Chem. Soc. (In press.)
11. LORING, H. S. Accuracy in the measurement of the activity of tobacco mosaic virus protein. Jour. Biol. Chem. **121**: 637–647. 1937.
12. ————. Properties of the latent mosaic virus protein. Jour. Biol. Chem. **126**: 455–478. 1938.
13. PIRIE, N. W., K. M. SMITH, E. T. C. SPOONER, and W. D. MCCLEMENT. Purified preparations of tobacco necrosis virus (Nicotiana virus II). Parasitol. **30**: 543–551. 1938.
14. ROSS, A. F. The concentration of alfalfa-mosaic virus in tobacco plants at different periods of time after inoculation. Phytopath. (In press.)
15. ————. The sulfur distribution in tobacco mosaic virus protein. Jour. Biol. Chem. **136**: 119–129. 1940.
16. STANLEY, W. M. Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus. Science **81**: 644–645. 1935. (Chemical studies on the virus of tobacco mosaic. VI. The isolation from diseased Turkish tobacco plants of a crystalline protein possessing the properties of tobacco-mosaic virus. Phytopath. **26**: 305–320. 1936.)
17. ————. Chemical studies on the virus of tobacco mosaic. VIII. The isola-

tion of a crystalline protein possessing the properties of aucuba mosaic virus. Jour. Biol. Chem. **117**: 325–340. 1937.

18. ———. The isolation and properties of tobacco ring spot virus. Jour. Biol. Chem. **129**: 405–428. 1939.
19. TILLMANS, J., and K. PHILIPPI. The carbohydrate content of the important proteins of foodstuffs and a colorimetric procedure for the determination of nitrogen-free sugar in protein. Biochem. Zeitschr. **215**: 36–60. 1929.
20. WEIMER, J. L. Studies on alfalfa mosaic. Phytopath. **24**: 239–247. 1934.
21. ZAUMEYER, W. J. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. Jour. Agr. Res. [U. S.] **56**: 747–772. 1938.

THE CONCENTRATION OF ALFALFA–MOSAIC VIRUS IN TOBACCO PLANTS AT DIFFERENT PERIODS OF TIME AFTER INOCULATION[1]

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It has been the practice in this laboratory to harvest virus-containing plants used as starting material in the purification of viruses about 4 weeks after inoculation. Attempts to isolate alfalfa-mosaic virus (*Marmor medicaginis* H.)[2] from tobacco plants that had been diseased for that length of time were, however, unsuccessful. Furthermore, it was found that the juice from such plants was practically noninfectious when rubbed on *Phaseolus vulgaris* L. var. Early Golden Cluster. This was contrary to the observation of Price (8), who obtained many lesions by rubbing with the juice of tobacco plants diseased with alfalfa mosaic. This contradiction suggested that the concentration of alfalfa-mosaic virus in tobacco plants might not be constant but might vary with the age of the plants. Consequently, a study was made of the virus content of diseased tobacco plants at different periods of time after inoculation. In the present paper are reported the results of the investigation, together with information on optimum conditions for the extraction of virus from the plant material and on dilution curves of the virus in juice from diseased plants.

EXPERIMENTAL

The virus used was obtained from W. C. Price and is believed to be identical with that designated by Zaumeyer as alfalfa-mosaic virus 1 (13). Holmes' necrotic-type tobacco (4), derived from Turkish tobacco (*Nicotiana tabacum* L.) and resistant to systemic infection by tobacco-mosaic virus, was used as host plant in most of the tests. This obviated possible contamination with tobacco-mosaic virus. The plants were grown in 4-inch pots in a greenhouse during the winter months. Twice weekly they were given light applications of a 0.06 per cent ammonium nitrate solution.

Virus activity was determined by the half-leaf method, described by Loring for tobacco-mosaic virus (5), using *Phaseolus vulgaris* var. Early Golden Cluster as a test plant. For reasons to be discussed later, juice was diluted 1:10 with 0.1 M phosphate buffer at pH 7.1 before being used for activity measurements.

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[1] A preliminary report of this work was presented before the American Phytopathological Society, at Columbus, Ohio, on December 28, 1939 (10).

[2] Nomenclature presented in *Handbook of Phytopathogenic Viruses*, by Francis O. Holmes, Burgess Publishing Company, Minneapolis, 1939.

Extraction of Virus

It was found in 1936 (11) that the addition of disodium phosphate to the macerated pulp of frozen tobacco-mosaic-diseased plants resulted in an appreciably higher virus content in the extracted juice, and since then disodium or dipotassium phosphate has been widely used in the extraction of viruses. It appeared desirable, therefore, to determine the effect of this material when used in the extraction of alfalfa-mosaic virus from tobacco plants. Diseased plants were ground in a meat grinder and the pulp divided into 2 portions. To one portion was added 3 per cent by weight of dipotassium phosphate in the form of a 50 per cent aqueous solution. After thorough mixing, both portions were allowed to stand at 4° C. for 1 hour. The juice was then removed from each by pressing through bandage gauze. A solution of dipotassium phosphate, equivalent in amount to that added to the pulp of one sample, was added to the juice from the nontreated portion. The juices were then centrifuged at 3000 r.p.m. at 4° C. for 30 minutes, diluted 1:10 with 0.1 M phosphate buffer, and tested for virus activity. The juice from the sample to which phosphate was added before extraction produced 1311 lesions on 35 half leaves, and the other caused 553 lesions on the opposite halves of the same leaves. The hydrogen-ion concentrations of the two juices were pH 6.97 and 7.15, respectively, while the 1:10 dilutions used for inoculation were both at pH 7.1. Since the two samples differed only in the manner in which the juices were extracted, the data indicate that the addition of phosphate before extraction resulted in more than a 2-fold increase in the virus activity of the extracted juice.

The foregoing experiment was repeated with a few modifications to determine whether or not the enhanced extraction of virus was attributable to the change in pH or to a specific action of the phosphate. Ground plant material was divided into 3 portions, A, B, and C. Dipotassium phosphate was added to A as in the preceding experiment, while B received no treatment, and C was adjusted to pH 6.6 by the cautious addition of 2 N sodium hydroxide. After the pulp had stood at 4° C. for 1½ hours, the juice was pressed from each preparation and then centrifuged. The juices thus extracted were tested for virus activity at a dilution of 1:10 in 0.1 M phosphate buffer. It may be seen from the results presented in table 1 that the addition of alkali to the pulp was as effective as the addition of phosphate, for both A and C were about 6 times as active as B, and a direct comparison of A with C revealed no difference in activity. The addition of 3 per cent by weight of dipotassium phosphate should result in a juice about 0.18 M with respect to phosphate, but, since appreciable quantities of insoluble phosphates are formed, the actual concentration would be less than 0.18 M. When such juice is diluted 1:10 with 0.1 M phosphate buffer, the phosphate concentration would be very near 0.1 M. As the samples differed only with respect to the method of extraction, it may be concluded that the addition

TABLE 1.—*Extraction of alfalfa-mosaic virus from tobacco plants*

Sample	Treatment of ground plant material	pH of juice	Concentration of phosphate buffer used for diluting	pH of inoculum	Lesions per half leaf[a]
A	K_2HPO_4 added before extraction	7.2	0.1 M	7.1	25.0
B	No treatment	5.6	0.1 M	7.1	3.4
A	K_2HPO_4 added before extraction	7.2	0.1 M	7.1	30.3
C	NaOH added before extraction	6.6	0.1 M	7.1	30.0
C	NaOH added before extraction	6.6	0.01 M	7.0	20.8
B	No treatment	5.6	0.01 M	6.9	3.3

[a] The samples paired vertically were rubbed on 24 or more opposite half leaves of *Phaseolus vulgaris* var. Early Golden Cluster.

of dipotassium phosphate before extraction of the juice results in an increase in the amount of virus extracted and that the effect is due to the increased pH and not to a specific effect of phosphate.

Dilution Curve

As an aid to the interpretation of activity data, dilution curves of the phosphate extract of tobacco plants diseased with alfalfa mosaic for different periods of time were determined. Young tobacco plants were inoculated with alfalfa-mosaic virus, and, at intervals of 5, 12, and 32 days, 6 plants were harvested and ground. Three per cent by weight of dipotassium phosphate was added, and the juice was extracted and centrifuged as previously described. Several different dilutions were made with 0.1 M phosphate buffer at pH 7.1 and the dilutions were compared on opposite half leaves with a 1:10 dilution of the same juice. In figure 1, relative numbers of lesions are plotted against dilution on a logarithmic scale, with the number produced by the 1:10 dilution arbitrarily assigned the value of 100. The curves for the 5-, 12-, and 32-day samples are markedly similar. The upper portions of the curves are unusual, for in that region dilution results in increased lesion formation. This phenomenon is probably caused by an inhibitor in the juice, for solutions of purified alfalfa-mosaic virus do not behave in a similar manner (9). Black (2) obtained the same type of curve with solutions of tobacco-mosaic virus mixed with insect juices.

At intermediate dilutions the number of lesions is roughly inversely proportional to the dilution. The range over which this is true is not great and varies somewhat with the various juices, being greater in those containing the most virus. Since the 1:10 dilution of each juice fell within this range, such a dilution was usually used in making activity measurements. At higher dilutions the dilution curve of alfalfa-mosaic virus again differs from that of most other viruses for which dilution-curve data are available, for the reduction in number of lesions is greater than the change in dilution, *i.e.,* the curve has a slope greater than 1. The same reduction was noted with purified alfalfa-mosaic virus (9). It also has been reported for certain

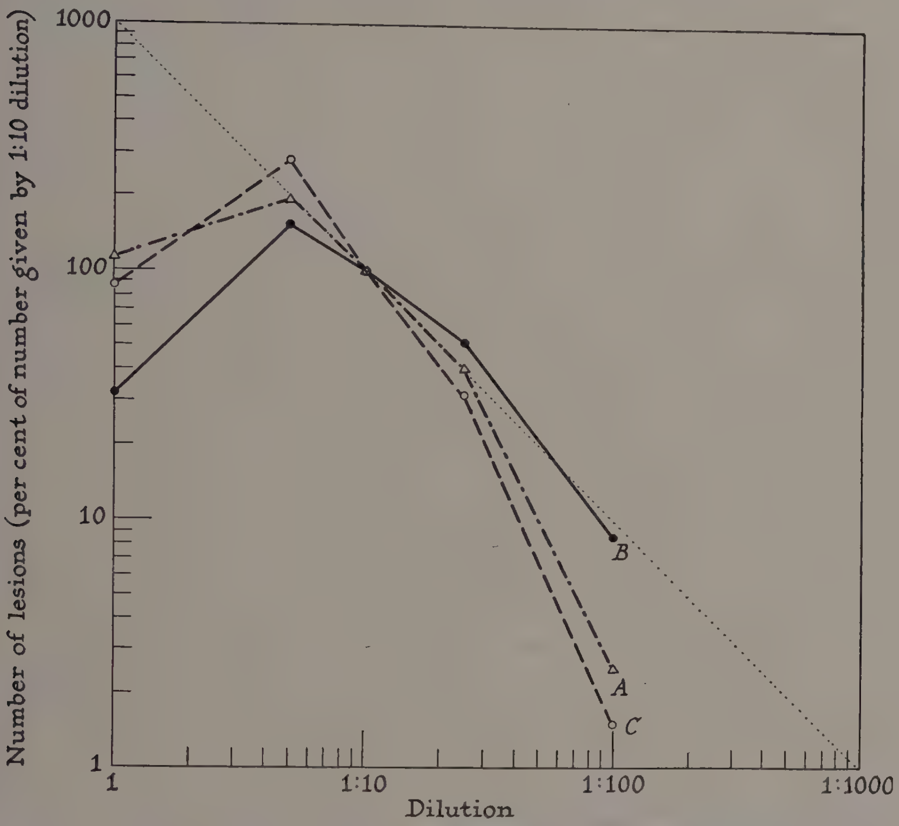

FIG. 1. Dilution curves of the juice obtained from tobacco plants diseased with alfalfa-mosaic virus for different periods of time. Juice was extracted after the addition of 3 per cent by weight of dipotassium phosphate and was approximately pH 7. The dilutions were made with 0.1 M phosphate buffer at pH 7.1. Each dilution of a given juice was rubbed on 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster, and a 1: 10 dilution of the same juice was rubbed on the opposite halves. Curves A, B, and C are the dilution curves of the juices obtained from plants diseased for 5, 12, and 32 days, respectively. The dotted line represents a theoretical curve of unit slope.

other viruses (1). In the present case it does not seem probable that the effect resulted from rapid inactivation of the virus in dilute solutions. Inoculations with the dilute solutions usually were made within 20 minutes after dilution; but, when greater periods of time elapsed, the several dilutions still possessed the same relative activities.

Virus Content of Plants at Intervals Following Inoculation

Different lots of young tobacco (Holmes' necrotic-type) plants were inoculated with alfalfa-mosaic virus at intervals of 2 to 24 days. Each plant, at the time of inoculation, possessed 4 leaves over 4 inches in length. An attempt was made to select uniform plants and to inoculate with virus solutions of approximately the same virus concentration each time. At a later date, 6 plants from each lot were harvested and the juice was extracted from each by the method previously described in which dipotassium phosphate was used. Each sample of juice was diluted 1:10 with 0.1 M phosphate buffer and compared for activity with a common control which consisted of a comparable dilution of the juice from plants diseased for 12 days. The virus activity of the juice, represented by curve A in figure 2, increased rapidly from about the 4th to the 11th or 12th day after inoculation of the plants, then decreased rapidly until the lesion count was as low as 1 per cent of the maximum. The data, presented as relative numbers of lesions, are probably fair estimates of the relative virus contents of the samples. The data in figure 1 indicate that the 1:10 dilution is within the suitable range for study, even with the older juices, and that the dilution curve of the juice of plants diseased for 12 days approximates a straight line with a slope of unity until a dilution of 1:100 is reached. Hence, with samples producing over 10 per cent as many lesions as the control, the relative number of lesions would be proportional to the actual virus content. The virus content of those samples producing less than 10 per cent as many lesions as the control probably would be somewhat greater than the amount indicated. The experiment was repeated several times and similar results were obtained each time. In one experiment, instead of using one control for all tests, samples inoculated at successive intervals were compared, *i.e.*, the 4-day sample was compared with the 6-day, the 6-day with the 8-day, etc. In another experiment the various samples were diluted, so that each gave approximately the same number of lesions. The curve obtained in each case was similar to that shown in figure 2, except that samples from plants inoculated for long periods of time appeared to be slightly more active than corresponding ones

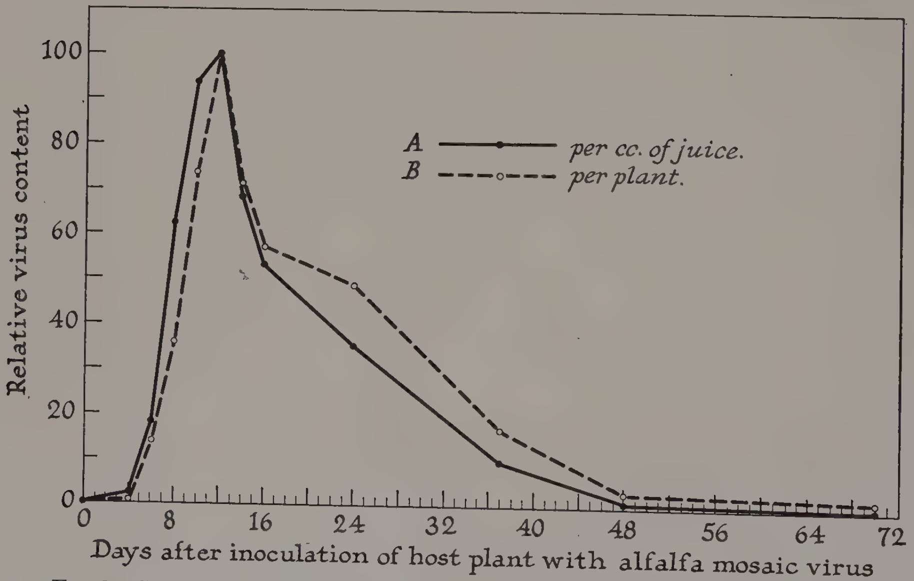

FIG. 2. Concentration of alfalfa-mosaic virus in tobacco plants diseased for different periods of time. Curve A represents the relative activity of the several juices. When the data were recalculated, taking into consideration the amount of juice obtained from each sample, the data represented by curve B were obtained. It is regarded as representing the relative amounts of virus present in the plants.

in figure 2. In still another experiment, the several samples of juice were ultracentrifuged and the pellets obtained were dissolved in 0.1 M phosphate buffer equal in volume to the original juice. These partly purified preparations were used for inoculations. The fact that the results did not differ significantly from those obtained with the juices indicates that the differences noted with the latter did not result from the presence of varying quantities of an inhibitor. It is possible that such a substance would not be separated from the virus by ultracentrifugation. However, experiments with virus purified by ultracentrifugation (9) provided no evidence of the presence of an inhibitor associated with the purified material.

The low virus content of the juice of the older plants is not because of any diluting effect of growth, for the virus concentration changes at a much more rapid rate than does the size of the plants. When the data presented in curve A, figure 2, were recalculated, taking into consideration the increase in size of plants, curve B was obtained, representing the relative virus content per plant. It can be concluded, therefore, that there is a rapid inactivation of alfalfa-mosaic virus in tobacco plants diseased for more than 12 days.

Tests for Presence of Neutralizing Antibodies

The rapid disappearance of virus activity after the 11th day is similar to that in some animal virus diseases and suggests that a defense mechanism in the tobacco plant may be causing an inactivation of virus in the plants. It seemed desirable, therefore, to examine diseased plants for the presence of neutralizing antibodies. Juice was extracted in the usual manner from tobacco plants diseased for 10 and for 30 days and from healthy plants.

TABLE 2.—*Results of tests for neutralizing antibodies in juice of diseased tobacco plants*

Sample	Description	Lesions per half leaf[a]		
		Undiluted	1: 5	1: 10
10	Juice from plants diseased 10 days	12.9	22.8	26.2
30	Juice from plants diseased 30 days	1.0	1.7	1.1
H	Juice from healthy plants	0.0	....	....
HSL	Supernatant liquid from UC[b] of H	0.0	....	....
10SL	Supernatant liquid from UC[b] of 10	1.0	....	....
30SL	Supernatant liquid from UC[b] of 30	0.1	....	....
10 + 30SL	Equal portions of 10 and 30SL	5.1	9.9	....
10 + HSL	Equal portions of 10 and HSL	8.8	15.9	....
10 + 30SL	Equal portions of 10 and 30SL	5.2	8.3	....
10 + 10SL	Equal portions of 10 and 10SL	7.9	11.8	....
10 + 30	Equal portions of 10 and 30	4.0	12.1	....
10 + 10SL	Equal portions of 10 and 10SL	4.5	16.1	....

[a] The samples paired vertically were rubbed on 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster. Dilutions were made with 0.1 M phosphate buffer at pH 7.1.

[b] The term UC should read ultracentrifugation.

Portions of each were ultracentrifuged for 1½ hours at 30,000 r.p.m., and the upper third of the supernatant liquids was drawn off. Five-cc. portions of the supernatant liquids or of the original juices were mixed with an equal volume of the juice from plants diseased for 10 days. After standing for 1 hour at 4° C., the mixtures were used for inoculation. It may be seen from table 2 that in no case did the mixing of juice result in a striking reduction of activity. The ultracentrifuged juice from the older diseased plants had a slightly greater effect than that of healthy plants or of plants diseased for 10 days. On other occasions, the mixing of juices caused a reduction in activity of about the same order of magnitude. The experiments failed to demonstrate definitely the presence of antibodies in such juices. If such substances are present in the plants, they may be almost entirely neutralized by previously formed virus or the method used may destroy or fail to extract them. The fact that the reduction in activity is no greater with undiluted juice than with a 1:5 dilution indicates that the effect is not due to the presence of different amounts of an inhibitor in the juices.

Location of Virus in Old Plants

Since all leaves of plants that have been diseased for several weeks show no symptoms, it seemed desirable to determine the location of the small amount of virus in such plants. The leaves of 3 tobacco plants that had been diseased for 46 days were removed and divided into 4 groups, according to their location on the plants. The juice was obtained from each in the manner already described, diluted 1:10 with 0.1 M phosphate buffer, and used for activity measurements. The results are tabulated in table 3,

TABLE 3.—*Location of virus in plants diseased for 46 days*

Location of leaves	Symptoms	Average weight of leaves per plant	pH of phosphate extract	Relative activity of juices[a]	Relative virus content[b]
		Grams		*Per cent*	*Per cent*
Upper 4 or 5 leaves plus tip	Faint mottling except in tips	3.3	7.00	98	24
Upper middle 3 or 4 leaves	Very faint mottling. Absent in some	5.8	6.67	100	43
Lower middle 6 or 7 leaves	No symptoms	24.8	6.82	17	31
Basal 4 or 5 leaves including inoculated leaves	No symptoms except for scattered primary lesions on inoculated leaves	15.7	6.73	2	2

[a] The figures in this column were obtained by dividing the number of lesions produced when a 1:10 dilution of the juice of the designated sample was rubbed on 24 or more half leaves of *Phaseolus vulgaris* var. Early Golden Cluster by the number produced when the control was rubbed on the opposite halves, and multiplying by 100. The control consisted of a 1:10 dilution of the sample to which the value of 100 is assigned.

[b] The values in this column were calculated from the data in columns 3 and 5.

together with notes on the appearance of the leaves included in each group. It is evident that about ⅔ of the total amount of virus was located in the upper leaves, representing about 18 per cent of the total leaf weight. Since the older leaves were once high in virus content but at the time of cutting were very low, it can be concluded that virus had disappeared from these leaves.

Although most of the virus in old plants is located in the upper leaves, it does not reach so high a concentration there as it does in the leaves of plants inoculated for shorter periods of time. Activity measurements were made in the usual manner on juices collected from the upper leaves of old diseased plants and from corresponding ones of younger plants. It may be seen from table 4 that the juices from plants diseased for 7 and for 10 days were about 2 and 3 times as active, respectively, as that from the top of older plants. Hence, virus is not only lost from the older tissues but it does not reach a very high level in those leaves formed after the 11th or 12th day following inoculation.

TABLE 4.—*Comparison of the activity of the juice from the upper leaves of tobacco plants diseased for long and for short periods of time*

Days after inoculation	Location of leaves	Dilution	Lesions per half leaf[a]	Relative activity
				Per cent
7	Upper 6 or 7 leaves	1:10	49.5	100
51	Upper 5 or 6 leaves	1:10	24.0	58
7	Upper 6 or 7 leaves	1:50	10.0	100
51	Upper 5 or 6 leaves	1:50	6.7	68
10	Upper 7 or 8 leaves	1:10	60.9	100
51	Upper 7 or 8 leaves	1:10	20.3	33
10	All 7 or 8 leaves	1:50	14.2	100
51	Upper 7 or 8 leaves	1:50	1.8	13

[a] The samples paired vertically were rubbed on 24 or more opposite half leaves of *Phaseolus vulgaris* var. Early Golden Cluster. Dilutions were made with 0.1 M phosphate buffer at pH 7.1.

Virus Concentration in Other Hosts

Since only Holmes' necrotic-type tobacco was used in the foregoing tests, it became of interest to determine whether or not the virus behaved in a similar manner in other hosts. Young plants of *Nicotiana glutinosa* L., Turkish tobacco, and necrotic-type tobacco were inoculated with alfalfa-mosaic virus, and, at intervals, 3 plants of each were harvested and the juices extracted from each in the usual manner. It may be seen from the results presented in table 5 that there was a more rapid decrease in the virus content of *N. glutinosa* than in that of the necrotic-type tobacco; but, after 33 days, the concentration in the two hosts was about the same. Turkish tobacco plants, diseased for 33 days, contained more virus than those of the necrotic type. In general, however, the virus behaved in approximately

TABLE 5.—*Concentration of alfalfa-mosaic virus in different hosts at intervals after inoculation*

Host	Days after inoculation	No. of lesions per half leaf[a]	Relative activity
			Per cent
Necrotic-type tobacco	11	30.7	100
Turkish tobacco	11	28.6	93
Necrotic-type tobacco	18	28.0	100
Turkish tobacco	18	23.7	85
Necrotic-type tobacco	33	15.4	100
Turkish tobacco	33	26.2	170
Necrotic-type tobacco	11	43.5	100
Nicotiana glutinosa	11	55.2	127
Necrotic-type tobacco	18	42.1	100
Nicotiana glutinosa	18	13.3	32
Necrotic-type tobacco	33	15.3	100
Nicotiana glutinosa	33	14.9	97

[a] The diluted juices of the samples paired vertically were rubbed on 24 or more opposite half leaves of *Phaseolus vulgaris* var. Early Golden Cluster. They were diluted 1:10 with 0.1 M phosphate buffer at pH 7.1.

the same manner in the 3 hosts and reached about the same concentration in each.

DISCUSSION

Tobacco plants inoculated with alfalfa-mosaic virus show the phenomenon referred to as recovery, for there is an acute stage of the disease followed by a chronic stage with much milder symptoms (3). This recovery differs in several respects from that in plants inoculated with tobacco-ring-spot virus (*Annulus tabaci* H. var. *virginiensis* H.) (6, 12). Plants that have recovered from tobacco ring spot show no symptoms except on those leaves where they had appeared before the recovery stage was reached. Plants recovered from alfalfa mosaic show symptoms only on the upper leaves, with the exception of the smallest leaves at the tip, and on the inoculated leaves. The symptoms on the upper leaves consist of a faint mottling, much milder than that apparent during the acute stage. The necrotic rings that sometimes appear on the inoculated leaf 4 to 7 days following inoculation do not entirely disappear. The systemic mottling characteristic of the acute stage gradually disappears and the leaves showing it become practically normal in appearance.

The changes in symptomatology of alfalfa mosaic are associated with the changes in virus concentration herein described. In some respects they resemble those observed by Price (6, 7) in plants diseased with tobacco ring spot; in other respects they differ. In both cases those leaves formed during the recovery period contain less virus and show milder symptoms than do leaves formed during the acute stage. In the case of tobacco ring spot, the virus content of those leaves showing symptoms during the acute stage remains fairly constant until the leaf turns yellow and drops from the plant. For a considerable period of time such leaves are higher in virus concentration than are the recovered upper leaves. In the case of alfalfa mosaic, the high concentration of virus reached during the acute stage is not maintained. As the symptoms disappear in the leaves, their virus content decreases. Consequently, the concentration of virus in the older leaves, once high, becomes less than that in the upper leaves. Hence, the recovery of tobacco plants appears to be similar to that characteristic of plants affected with tobacco ring spot, with the added condition that alfalfa-mosaic virus is unstable *in vivo.* Prior to the 11th day, virus is produced at a more rapid rate than it is inactivated. The rapid decrease in the virus content after the 11th day may be due to a sharp decline in the rate of virus multiplication similar to that occurring with tobacco ring spot, while the rate of inactivation continues and exceeds the rate of multiplication, resulting in a net loss of virus. The cause of the inactivation is not known. The results of the few tests for neutralizing antibodies in the juice of recovered plants did not demonstrate their presence. It is possible that the decrease is due to a thermal inactivation *in vivo,* for the virus is unstable at greenhouse temperatures and the virus content of diseased plants appears to be less during hot summer months than in winter, when the temperatures were maintained at 20–24° C. (9).

SUMMARY

When young tobacco plants are inoculated with alfalfa-mosaic virus, the virus content of the plant increases from about the 4th to the 12th day after inoculation, then decreases rapidly. The juice from plants diseased for 48 days or longer may be less than 1 per cent as active as that from plants diseased for only 12 days. In the older plants, most of the virus is located in the upper leaves, which show only a faint mottling; but, even in such leaves, the concentration is much less than that reached about 12 days after inoculation. The older leaves, which once contained a high concentration of virus, contain very small amounts of virus and show no symptoms. Disappearance of symptoms is associated with loss of virus in such plants. It is suggested that tobacco plants, inoculated with alfalfa-mosaic virus, undergo a type of recovery similar to that exhibited by plants diseased with tobacco-ring-spot virus, and that the two phenomena differ in that the former virus is unstable *in vivo.*

The addition of dipotassium phosphate to ground diseased tobacco plants before pressing out the juice more than doubles the amount of virus extracted. With juice obtained in such a manner, dilution over a limited range results in an increase in the number of lesions produced. At medium dilutions, the number of lesions produced is inversely proportional to the dilution, but, at higher dilutions, the decreases in the number of lesions produced are much greater than would correspond to the changes in dilution.

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LITERATURE CITED

1. BALD, J. G. The use of numbers of infections for comparing the concentration of plant virus suspensions. II. Distortion of the dilution series. Ann. Appl. Biol. **24**: 56–76. 1937.
2. BLACK, L. M. Inhibition of virus activity by insect juices. Phytopath. **29**: 321–337. 1939.
3. ————, and W. C. PRICE. The relationship between viruses of potato calico and alfalfa mosaic. Phytopath. **30**: 444–447. 1940.
4. HOLMES, F. O. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopath. **28**: 553–561. 1938.
5. LORING, H. S. Accuracy in the measurement of the activity of tobacco mosaic virus protein. Jour. Biol. Chem. **121**: 637–647. 1937.
6. PRICE, W. C. Acquired immunity to ring-spot in *Nicotiana*. Contrib. Boyce Thompson Inst. **4**: 359–403. 1932.
7. ————. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopath. **26**: 503–529. 1936.
8. ————. Thermal inactivation rates of four plant viruses. Arch. ges. Virusforsch. **1**: 373–386. 1940.
9. ROSS, A. F. Purification and properties of alfalfa mosaic virus protein. May, 1941. (In press.)
10. ————, and W. M. STANLEY. Properties and purification of alfalfa-mosaic virus. (Abstract.) Phytopath. **30**: 20–21. 1940.
11. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. VI. The isolation from diseased Turkish tobacco plants of a crystalline protein possessing the properties of tobacco-mosaic virus. Phytopath. **26**: 305–320. 1936.
12. WINGARD, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. [U. S.] **37**: 127–153. 1928.
13. ZAUMEYER, W. J. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. Jour. Agr. Res. [U. S.] **56**: 747–772. 1938.

STUDIES ON MOSAIC OF COWPEAS, VIGNA SINENSIS[1]

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INTRODUCTION

In the spring of 1938, a mosaic disease of cowpeas (*Vigna sinensis*) caused severe dwarfing of the plants in certain experimental plots on the Oklahoma Agricultural and Mechanical College farm. The disease appeared to spread from a few infected plants until all in these plots were infected.

The first report of cowpea mosaic known to the writer is that made by Elliott (2) in Arkansas in 1921. By 1929, records of the disease were issued to the Plant Disease Reporter from Oklahoma, Louisiana, Indiana, Georgia, Iowa, Mississippi, Kansas, and New Jersey.

Smith (15) was the first to report insect and manual transmission of cowpea mosaic. He stated that the bean leaf beetle, *Ceratoma trifurcata*, is an efficient vector of the virus. Gardner (5) in 1927 reported seed transmission of the virus in the varieties Progressive White and Arlington.

Chester (1) estimated the loss at 30 per cent in several fields in Oklahoma in the season of 1939 and stated that the disease is seen frequently at various points in the State. Preliminary investigations demonstrated that the mosaic virus was seed-borne in the varieties New Era and Whippoorwill.

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[1] The writer wishes to acknowledge gratefully his indebtedness to Dr. K. Starr Chester and Dr. A. L. Pierstorff for helpful advice during the preparation of this manuscript.

Studies were undertaken to determine varietal susceptibility, host range, properties, and methods of transmission of the virus. The experiments described were undertaken at The Ohio State University in 1938, 1939, 1940. Insect transmission experiments were conducted in plantings at Old Fort, Ohio, in the summer of 1939.

SYMPTOM EXPRESSION

Seed samples of several important varieties of cowpeas were obtained from leading seedsmen and from diseased plants of the New Era and Whippoorwill varieties grown on the Oklahoma A. and M. College farm. Seeds from these samples were planted in the greenhouse and the plants were carefully examined for symptoms of disease when the first compound leaves

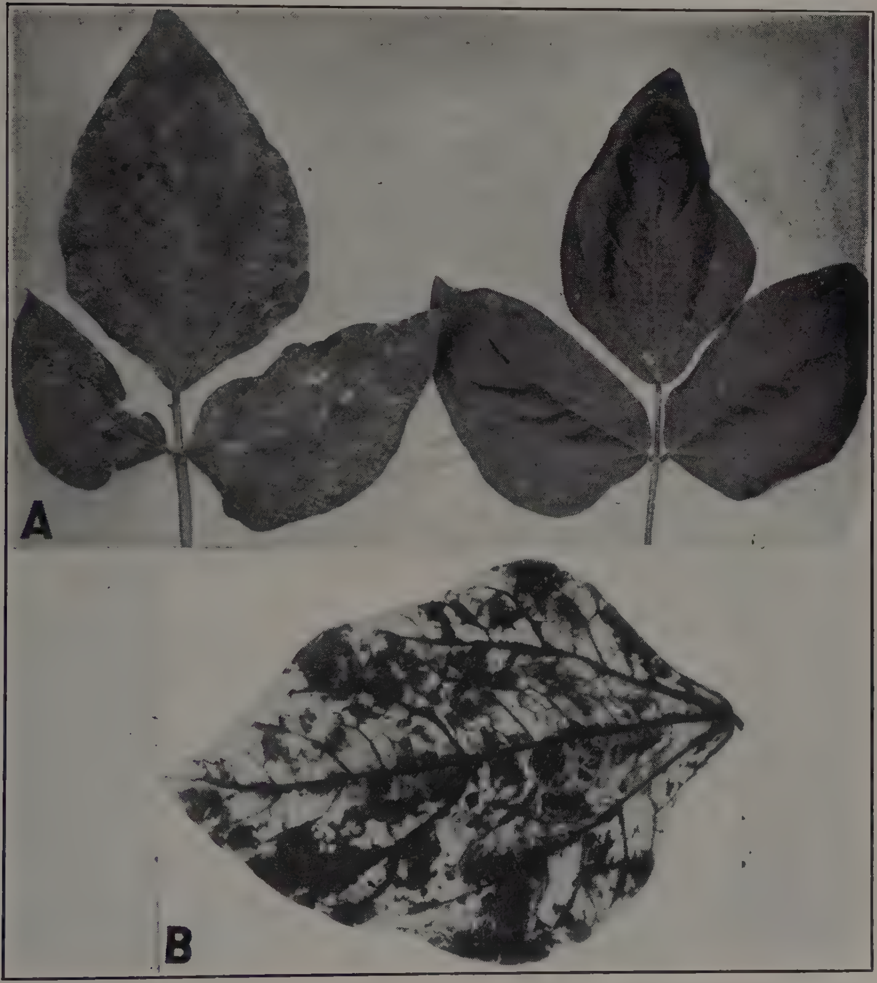

FIG. 1. A. Diseased cowpea leaf, showing characteristic symptoms of mottling. B. Mosaic-mottled cowpea leaflet after staining with potassium iodide. Dark stained areas illustrate the presence of starch; nonstained areas are starch-free.

were fully developed. The seeds obtained from the seedsmen were free from virus infection; however, those obtained from the mosaic infected plants carried the virus in 5 per cent of the seeds.

Primary symptoms of mosaic usually appear in the simple leaves of plants grown from infected seeds. Vein-clearing in most cases is the first noticeable symptom in the developing leaves. Associated with or following vein-clearing, a typical mottling of light and dark green is evident. Sometimes, one or both of the simple leaves may exhibit mottling with occasional curled or ruffled edges.

There is fundamentally little difference in the characteristic symptoms produced in the first compound leaves or the succeeding ones in plants infected from seed or those secondarily infected. Irregular patches of a lighter green are found interspersed with the normal green (Fig. 1, A). These patches often are found as elongated blotches running parallel with the veins of the leaflet. Accompanying the mottling there is frequently a slight convex cupping or arching of the leaflets. Plants grown from infected seed exhibit shortened internodes, prolepsis, and the petioles are frequently twisted and shorter than those on healthy plants. As a result of the systemic invasion the vegetative phase is prolonged, the plants are delayed in maturity, and are still green at frost, after healthy plants have ceased growing and the seeds have ripened.

TRANSMISSION

Literature shows but few records of successful artificial transmission of cowpea mosaic. Smith (15) transmitted the disease by rubbing the leaves of diseased and healthy plants together and by needle inoculation. Chester (1) states that it can be transmitted mechanically but not with 100 per cent regularity.

By Mechanical (Manual) Inoculation

In transmission studies, the method of Rawlins and Tompkins (13), whereby carborundum powder was used as an abrasive with the virus extract, gave a high percentage of infection. The virus was easily transmitted from diseased to healthy plants by pinching off a leaflet from a diseased plant by means of two pot labels, and, after crushing the leaflet between the labels, applying the macerated pulp directly to the leaf surface of a healthy suscept (8).

The time elapsing between inoculation and the appearance of symptoms varied from 8 to 12 days. As noted by Elmer (4), the symptom expression is directly correlated with the vigor and rate of growth of the plants; that is, a vigorously growing plant exhibits more striking symptoms and does so in a shorter period of time following inoculation than does one of retarded growth. Readily recognizable symptoms develop only on foliage appearing subsequent to inoculation, and greenhouse conditions during the winter months may retard symptom expression. Exposing inoculated plants to light from 200-watt Mazda lamps from 3:00 p.m. to 12:00 p.m. shortened the time of symptom expression.

By Insects

Smith (15) reported that the bean leaf beetle is a definite and efficient carrier of the cowpea-mosaic virus. Extensive inspections of cowpeas at Stillwater, Oklahoma, in 1938, and of beans and cowpeas in plantings near Old Fort, Ohio, in 1939, indicate that the bean leaf beetle does not habitually feed on the cowpea plant in large numbers in these regions and, therefore, is unlikely to be a frequent vector in the spread of this disease. In early plantings of cowpeas, the writer has observed rapid spread of mosaic without the appearance of any chewing insect. Spread of the disease appears to be correlated with the aphid population.

TABLE 1.—*Results of cage experiments on insect transmission of cowpea mosaic, Old Fort, Ohio, 1939*

Insects used	No. plants inoculated	No. plants infected	Percentage infection
Black aphid (Sp. undet.)	8	8	100
Potato aphid (*Macrosiphum solanifolii*, Ashm.)	10	6	60
Melon aphid (*Aphis gossypii* Glov.)	12	12	100
Pea aphid (*Macrosiphum pisi* Kolt.)	7	5	70
Tarnished plant bug (*Lygus pratensis* L.)	6	0	0
Striped cucumber beetle (*Diabrotica vittata* Faba.)	4	0	0
Mexican bean beetle (*Epilachna corrupta* Muls.)	4	0	0
Bean leaf hopper (*Empoasca fabae* Le. B.)	12	0	0

Elmer (3, 4) states that aphids and mealy bugs, *Pseudococcus maritimus* Ehr., carried the mosaic virus from potato, cucumber, and crookneck squash to beans and cowpeas.

In preliminary investigations in the greenhouse, the writer used the peach aphid, *Myzus persicae* Sulz,[2] and the potato aphid, *Macrosiphum solanifolii* in caged transmission experiments. The results indicate that both of these species are efficient vectors of the virus.

During the season, a collection was made of the more common species of aphids on cowpeas. The first aphid observed was a black species (undetermined), closely resembling the black bean aphid, *Aphis rumicis* L. Other species included the potato aphid, the melon aphid, *Aphis gossypii*, and the pea aphid, *Macrosiphum pisi*. In separate experiments, a number of each of these species were caged on diseased cowpea plants for 2 days. They were then transferred to caged healthy plants by removing a portion of the diseased plants containing the aphids and placing it at the base of the healthy plant.

The results (Table 1) were so striking as to leave little doubt of the ability of these insects to transmit the virus. After 7 days, typical symptoms of mosaic appeared in the majority of transfers. Since ability to transmit the virus is not necessarily confined to certain species of aphids, it is probable that other species not tested also would prove efficient vectors.

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[2] Aphids were supplied by the Department of Entomology, The Ohio State University.

Many other species of insects were observed on cowpeas, the more common ones being the tarnished plant bug, *Lygus pratensis,* the striped cucumber beetle, *Diabrotica vittata,* Mexican bean beetle, *Epilachna corrupta,* and the bean leaf hopper, *Empoasca fabae.* Transmission experiments with these insects yielded negative results.

By Seed

In order to study seed transmission of the mosaic virus in varieties of cowpeas that showed different degrees of susceptibility to the disease in the field, seeds harvested from these varieties were planted in the greenhouse and observations were made on the percentage of diseased seedlings in the respective varieties. When the plants had developed the first compound leaves, they were carefully examined for mosaic symptoms. The results of this test are given in table 5.

A study of the table will reveal a correlation between varietal susceptibility and the percentage of infected seeds produced. The highly susceptible varieties produced more virus-infected seeds than the slightly susceptible or somewhat resistant varieties.

PROPERTIES OF THE VIRUS

A series of experiments was undertaken to determine the physical properties of the virus after the writer had found that the virus could be transmitted readily from plants grown from infected seeds. For this purpose diseased seedlings were crushed and the sap extracted. After treatment, the juice was rubbed into the leaves of healthy New Era plants dusted with carborundum powder.

Aging *in vitro*

Extract was stored in a cotton stoppered Erlenmeyer flask at room temperature, ranging from 20 to 25 degrees Centigrade. After different intervals of time, juice from the flask was used for inoculation to healthy suscepts. The results of these tests are shown in table 2. The extract was infective after aging *in vitro* for periods up to and including 48 hours.

TABLE 2.—*Effect of aging cowpea-mosaic virus extract in vitro on infectivity*

Time aged (hours)	Plants inoculated	Plants infected
Control	24	22
12	12	5
24	12	3
48	12	1
72	12	0
96	12	0

Thermal Inactivation

Resistance to heat was determined by placing 3 cc. of the extract in thin test tubes, heating in a water bath at the desired temperature for 10 minutes, cooling rapidly, and then testing its infectivity by inoculation to healthy New Era plants. The results of several experiments are given in table 3. Infection was obtained from extract heated at various temperatures up to 72° C., but was not obtained from sap heated to 75° C.

TABLE 3.—*Effect of heating cowpea-mosaic virus extract at different temperatures on infectivity*

Temperature (in degrees Centigrade) for 10 minutes	No. plants inoculated	No. plants infected
Control	12	9
60	12	9
63	12	6
66	12	5
69	12	5
72	12	3
75	12	0
78	12	0

Effect of Dilution

To determine the effect of dilution, extract was diluted with sterile water and tested for infectivity by manual inoculations to healthy New Era plants. The results are shown in Table 4.

TABLE 4.—*Effect of diluting cowpea-mosaic virus extract on infectivity*

Dilution	No. plants inoculated	No. plants infected
Control	24	22
1: 5	12	11
1: 10	12	11
1: 100	12	7
1: 500	12	3
1: 1000	12	3
1: 1500	12	0
1: 10000	12	0

EFFECT OF THE VIRUS ON STARCH SYNTHESIS

Leaves from diseased plants that had been exposed to moderate sunlight for a period of 5–8 hours were tested for starch with potassium iodide by the method described by Meyer and Anderson (10). The leaves displayed patterns corresponding to the green and yellowish-green areas in the leaves; indicating that the synthesis of starch had been inhibited in the leaf tissues where chlorophyll was deficient. The yellow-green areas contained little starch and did not stain, while the green areas, which stained dark-purple, contained an abundance of starch (Fig. 1, B).

VARIETAL SUSCEPTIBILITY

Gardner (5) mentioned a number of varieties of cowpeas (Early Red, Early Black, Whippoorwill, Iron, Red Ripper, Arlington, Clay, Conch, Columbia, Groit, and the related species *V. catjang*), which were infected during the growing season of 1927 in Indiana, after the disease had spread by natural inoculation agents from the infected Progressive White variety.

Some idea of the relative susceptibility of the different varieties being tested was gained from field experiments. Seeds of each of the varieties referred to in table 5, were planted in successive rows 150 ft. long with 36 inches between the row. The varieties New Era and Whippoorwill were alternately planted in the 3rd, 6th, 11th, and 16th rows. When the plants had developed sufficiently, and under conditions that may have been unfavorable for some varieties, to exhibit symptoms, each variety was carefully examined for primary symptoms arising from seed-borne infections. Such infections were found only in the New Era and Whippoorwill varieties. After several weeks, an increased number of diseased plants, probably due to secondary infections by naturally present insect vectors, was observed; by July 15, nearly 100 per cent infection was evident in the highly susceptible varieties. All plants were grown to maturity and rec-

TABLE 5.—*The relative susceptibility of cowpea varieties to mosaic and the percentage of seed transmission*

Variety	Seed Transmission			
	No. seeds planted	No. seeds germinated	No. seedlings infected	Percentage of infection
	Class 1. Highly Susceptible			
New Era[a]	1000	925	49	5.0
Whippoorwill[a]	1000	827	38	4.5
Brabham	1000	829	17	2.0
Groit[b]	....	....	......	......
Rice Pea	90	29	2	6.8
Lady Pea[b]	....	....	......	......
Cotton-Patch[b]	....	....	......	......
	Class 2. Moderately Susceptible			
Columbia[b]	....	....	......	......
Conch	5570	291	16	5.0
Early Ramshorn Blackeye	1000	770	26	3.0
	Class 3. Susceptible			
Clay	210	164	5	3.0
Victor[b]	....	....	......	......
Brown Sugar Crowder	400	364	16	4.0
Catjang[b]	....	....	......	......
	Class 4. Slightly Susceptible			
Cream Crowder	1130	1031	21	2.0
Red Ripper	1000	600	0	0.0
Taylor	1000	803	27	3.0
Iron	190	141	2	1.0
Black	1051	712	0	0.0

[a] Indicated original seed-borne infections.
[b] Data on seed transmission were not kept on these varieties.

ords kept on the relative severity of the mosaic as displayed in the external symptoms of each variety (Table 5).

Prolepsis and dwarfing, when either occur in conjunction with other symptoms, are regarded as indications of extreme susceptibility. These characters are not evident in all varieties, and the only symptoms present is the mosaic pattern in the younger leaves.

Four groups were recognized by the writer in an arbitrary classification as exhibiting relative susceptibility to mosaic. This grouping was based on external symptoms and the general vigor of the plants in the row.

Class 1. Highly susceptible: Plants were severely dwarfed and showed marked abortion of flowers in early infections. The leaflets were malformed and prominently mottled.

Class 2. Moderately susceptible: Plants were definitely dwarfed. Leaf symptoms were not as prominent as in class 1.

Class 3. Susceptible: Plants of this class showed less effects of mosaic infection than those in class 2. There was little stunting of plants or reduction in yield of pods. Mosaic symptoms were evident in the younger leaves.

Class 4. Slightly susceptible (somewhat resistant): Plants were lightly mottled in the younger leaves only. The plants grew vigorously and apparently were not injured by the virus.

TRANSMISSIBILITY TO OTHER SUSCEPTS

Since the suscept relationship is a vital factor in the study and control of viruses, an attempt was made to transfer the mosaic virus of cowpeas to a number of species of Leguminosae, as well as a number of other species that have proved susceptible to mosaic viruses.

Inoculations were made by applying macerated tissue from diseased cowpea plants to the leaves of the healthy suscepts by means of sterile pot labels after sprinkling carborundum powder on the suscept leaves. The pea aphid also was used in attempts to transmit the virus, where this insect would transfer readily.

In repeated tests, the Lima bean (*Phaseolus lunatus macrocarpus* Benth.) variety Wood's Prolific, proved susceptible to infection. Mild symptoms were visible in 8 to 10 days following inoculation. Vein clearing was the first noticeable symptom, followed by mild-mosaic mottling in the young leaves.

Attempts to infect the following species were negative: *Vicia faba* L., *Pisum sativum* L., *P. sativum* var. *arvense* L., *Lathyrus odoratus* L., *Phaseolus vulgaris* L., *P. aureus* Roxb., *P. lunatus* L. var. Fordhook Bush Lima, *Lycopersicon esculentum* Mill., *Solanum tuberosum* L., *Petunia hybrida* Hort, *Datura stramonium* L., *Capsicum annuum* L., *Zinnia elegans* Jacq., *Cucumis sativus* L., *Nicotiana tabacum* L., *N. glutinosa* L.

These results are not conclusive, as this experiment was performed in late winter when conditions were not entirely favorable for the expression of symptoms.

DISCUSSION

The reports of the occurrence of the mosaic in cowpeas (15, 5, 2, 1), probably deal with the same disease here reported, since it has been shown that the virus is seed-borne and no other virus has been reported as causing a naturally incident infection of cowpeas.

Harter (6) and McClintock (9) studied a mosaic of Lima beans and found that the causal virus also produced local necrotic lesions on the Black and Blackeye cowpeas (*Vigna sinensis*). Harter (6) proposed that this virus be designated a Lima-bean strain of the cucumber virus because of its similarity to the latter. In a parallel experiment he inoculated the Black cowpea with juice from mosaic-diseased celery, cucumber, and Lima bean and obtained numerous lesions in all cases. Wellman (17) was unable to transfer a mosaic virus from celery to cowpeas with the melon aphid. Common bean mosaic, according to Reddick and Stewart (14), would not produce infection on Blackeye cowpeas. Pierce (11) was unable to produce infection on cowpeas with bean virus 1, bean virus 2, alfalfa virus 2, and tobacco virus 1. The writer was unable to cause infection on cowpeas with tobacco virus 1.

Zaumeyer and Wade (19) related various legume mosaics to bean mosaic, and, in cross inoculations, were unable to infect cowpeas with pea mosaic virus 2, white-clover mosaic, white-sweet-clover mosaic, alfalfa mosaic, red-clover mosaic, and common bean mosaic. Zaumeyer (20) found that alfalfa-mosaic virus 1, virus 1A, and virus 1B, caused local lesions on cowpeas. The local lesions produced by alfalfa-mosaic viruses on cowpeas are, as pointed out by Price (12), quite similar to those produced by various strains of cucumber-mosaic virus.

Elmer (4) was unable to transfer a mosaic virus of cowpeas by manual inoculation, but found that aphids transferred the virus under greenhouse conditions. He was able to demonstrate that cowpeas could be infected with viruses from potato and cucumber with viruliferous aphids, while mealy bugs transmitted viruses from crookneck squash, eggplant, and soybeans to cowpeas. Elmer (4) did not describe the symptoms of diseases on the various suscepts in his cross-inoculations nor did he specify the source of the cowpea virus. It is not known whether he was dealing with the mosaic virus reported in this paper.

Stubbs (16) was unable to secure infection on the Iron variety of cowpea with pea virus 1, pea virus 2, pea virus 2B, pea virus 2C, and tobacco ring-spot virus. Wingard (18) secured necrotic lesions on Blackeye cowpea leaves with the tobacco ring-spot virus. Pierce (11) found that tobacco ring-spot virus produced local necrotic lesions followed by systemic infection, which, in nearly all cases, caused death of Groit cowpea plants. Tobacco ring-spot also will infect cucumber.

It has been demonstrated that celery mosaic, cucumber mosaic, and Lima bean mosaic are closely related diseases and apparently caused by strains of the same virus. None of the following legume viruses appear to infect cowpeas; bean virus 1, bean virus 2, pea virus 1, white-clover-mosaic virus, white-sweet-clover-mosaic virus, and red-clover-mosaic virus. Alfalfa viruses 1, 1A, 1B, appear to bridge the gap between the legume viruses and the cucumber-virus group, since they cause local lesions on leaves of beans, broadbeans, and cowpeas. Alfalfa mosaic viruses 1A and 1B produce systemic mosaic infection in cucumber, zinnia, and petunia.

Price (12) obtained localized necrotic lesions on the leaves of 8 common varieties of cowpeas when inoculated with Porter's cucumber-mosaic virus. Johnson's cucumber-mosaic virus produced similar results. Price obtained 2 strains (strains y1, y2) of the original cucumber-mosaic virus that caused systemic infection in the Blackeye cowpea. The symptoms produced by these strains are apparently similar to those by the cowpea-mosaic virus herein described. Price's cowpea strain of cucumber mosaic is the strain classified by Holmes (7) as *Marmor cucumeris* var. *vignae,* and has not been found occurring naturally on cowpeas.

SUMMARY

The mosaic disease of cowpeas was first reported from Arkansas in 1921. Since then it has been found in Indiana, Louisiana, Oklahoma, Georgia, Mississippi, Iowa, Kansas, and New Jersey.

The highly susceptible varieties exhibit the most striking symptoms. A dwarfed slender growth with a tendency toward excessive branching is associated generally with mosaic.

Cowpea mosaic can be transmitted by ordinary manual methods of inoculation, but not with 100 per cent regularity. Carborundum powder aided in transmission of the virus when it was used as an abrasive with sap extracted from diseased plants. Vigorously growing plants exhibit symptoms in a shorter period than slowly growing plants.

The highly susceptible varieties commonly carry the virus in about 5 per cent of the seeds from infected plants.

The virus was transmitted by the following species of aphids: *Macrosiphum solanifolii, M. pisi, Aphis gossypii, Myzus persicae,* and a black aphid (species undetermined). The bean leaf hopper, tarnished plant bug, Mexican bean beetle, and the striped cucumber beetle failed to transmit the virus.

The virus was infective after aging *in vitro* for periods up to and including 48 hours, but was not infective after this period. The thermal inactivation point ranges between 72 and 75° C. The virus was not infective in dilutions greater than 1:1000.

Starch synthesis is inhibited in the yellowish-green areas in the leaves. When the leaves were stained with potassium iodide, the green areas, which stained deep-purple, contrasted sharply with the yellowish-green areas that did not stain.

Cowpea mosaic virus was transmitted to Lima bean by manual inoculation and by the pea aphid. Attempts to infect the following species were unsuccessful: *Vicia faba, Pisum sativum, P. sativum* var. *arvense, Lathyrus odoratus, Phaseolus vulgaris, P. aureus, P. lunatus* var. Fordhook Bush Lima, *Lycopersicon esculentum, Solanum tuberosum, Petunia hybrida, Datura stramonium, Capsicum annuum, Zinnia elegans, Cucumis sativus, Nicotiana tabacum* and *N. glutinosa.*

The relation of the cowpea-mosaic virus to several common mosaic viruses is discussed.

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LITERATURE CITED

1. CHESTER, K. STARR. Some important diseases in Oklahoma. Pl. Dis. Rptr. **23**. 1939.
2. ELLIOTT, J. A. A mosaic of sweet and red clover. Phytopath. **11**: 146–148. 1921.
3. ELMER, O. H. Mosaic cross-inoculation and insect transmission studies. Science (n. s.) **56**: 370–372. 1921.
4. ————. Transmissibility and pathological effects of the mosaic disease. Iowa Agr. Exp. Stat. Bull. 82: 39–91. 1925.
5. GARDNER, M. W. Seed transmission of cowpea mosaic. Ind. Acad. Sci. Proc. **43**. 1927.
6. HARTER, L. L. Mosaic of lima beans, *Phaseolus lunatus macrocarpus*. Jour. Agr. Res. [U. S.] **56**: 895–906. 1938.
7. HOLMES, F. O. A handbook of phytopathogenic viruses. Burgess Publishing Co. (Minneapolis). 1939.
8. JENSEN, J. H. Isolation of yellow-mosaic viruses from plants infected with tobacco-mosaic. Phytopath. **23**: 964–974. 1933.
9. MCCLINTOCK, J. A. Lima bean mosaic. (Abstract) Phytopath. **7**: 60–61. 1917.
10. MEYER, B. S., and D. B. ANDERSON. Laboratory plant physiology. Edwards Brothers Inc. (Ann Arbor). 1937.
11. PIERCE, W. H. Viruses of the bean. Phytopath. **24**: 87–115. 1934.
12. PRICE, W. C. Isolation and study of some yellow strains of cucumber mosaic. Phytopath. **24**: 743–761. 1934.
13. RAWLINS, T. E., and C. M. TOMPKINS. The use of carborundum as an abrasive in plant virus inoculations. (Abstract) Phytopath. **24**: 1147. 1934.
14. REDDICK, D., and V. B. STEWART. Varieties of beans susceptible to mosaic. Phytopath. **8**: 530–534. 1918.
15. SMITH, C. E. Transmission of cowpea mosaic by the bean leaf beetle. Science (n. s.) **60**: 268. 1924.
16. STUBBS, M. W. Viroses of the garden pea, *Pisum sativum*. (Abstract) Phytopath. **26**: 108–109. 1936.
17. WELLMAN, F. L. Identification of celery virus 1, the cause of southern celery mosaic. Phytopath. **24**: 695–725. 1934.
18. WINGARD, S. A. Hosts and symptoms of ring-spot, a virus disease of plants. Jour. Agr. Res. [U. S.] **37**: 127–153. 1933.
19. ZAUMEYER, W. J., and B. L. WADE. The relationship of certain legume mosaics to bean. Jour. Agr. Res. [U. S.] **51**: 715–749. 1935.
20. ————. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. Jour. Agr. Res. [U. S.] **56**: 747–772. 1938.

BRAZILIAN BEAN VARIETIES AS PLANT INDICATORS FOR THE TOBACCO-MOSAIC VIRUS

K. SILBERSCHMIDT AND M. KRAMER

(Accepted for publication August 19, 1940)

INTRODUCTION

Price (6) already has reported that some varieties of garden beans (*Phaseolus vulgaris*) develop local necrotic lesions when the upper surface of the cotyledon leaves is rubbed with juice containing the virus of the ordinary tobacco mosaic (Johnson's tobacco virus 1).

Experiments performed by Price showed that lesions produced on certain bean varieties are useful in indicating relative concentrations of virus in samples of juice from mosaic-affected plants. There were obtained specially good results with the bean varieties Scotia and Early Golden Cluster.

In our studies on the significance of the tobacco-mosaic disease in the State of São Paulo (7) we have obtained good results in the past 2 years from beans of the Scotia variety, kindly supplied by Dr. W. C. Price. Nevertheless, we tried to find out whether other varieties also might serve as indicators of virus concentration in mosaic-virus-containing liquids. Therefore, we performed experiments on the behavior of 73 other bean varieties, obtained in great part through the kindness of Dr. C. A. Krug and L. A. Nucci of the Instituto Agronomico do Estado at Campinas.

MATERIAL

We used in our comparative studies a collection of bean varieties, part of which were bought on the market in São Paulo. Naturally, the varieties thus obtained represent samples of commercial value and of frequent consumption in the State of São Paulo. Of the varieties mentioned, without designation by number (Table 1), the following samples belong to this group: Preto S. Paulo, Preto Rio Grande, Leite, and Chumbinho.

Most of the varieties used in our studies were obtained through courtesy of the Department of Genetics, Instituto Agronomico do Estado, at Campinas, and consisted of 69 samples, the greater part of which represent Brazilian varieties, cultivated many years for genetical purposes in Campinas. To the collection also belonged a few foreign varieties, received in recent years at the Instituto Agronomico. We mention here first the following North American varieties, supplied in 1937 to the Instituto Agronomico by the University of Idaho and since multiplied in Campinas: Robust No. 253, Great Northern No. 249, Idaho Refugee No. 252.

The varieties Stringless Greenpod No. 199 and Canadian Wonder No. 204 were received in 1936 in Campinas, from the Department of Agriculture, Brisbane, Australia.

The following varieties were received in 1936 from the Agricultural Department of Costa Rica: Jamaiqueño No. 212, Higuerilla Blancos No. 221, Blancos o Lechillas No. 234 and Ahumados del Coyol No. 241. Finally, we still have to explain briefly the descent of the greatest part of the varieties of the collection, *e.g.*, those considered Brazilian. Some of them, as the samples named Mulatinho Nos. 1–4, Bico de Ouro 65, Enxofre 111 and Manteiga 9 are varieties cultivated here on a large scale, being of a great economic importance. Other varieties, as Porto Alegre 34, Chita 121 and 122, Fosco 124, from Brazilian agricultural experiment stations, are of a more limited economic interest.

In the collection there are still a few varieties with foreign names, as Long Fellow 20, Thousand-to-one 21, Byington 18, that are, of course, originally imported from other countries, but are now already acclimated in São Paulo.

In table 1, all the varieties received from Campinas are designated by the numbers given them in the collection of the Department of Genetics.

TABLE 1.—*The behavior of Brazilian and foreign bean varieties in presence of tobacco-mosaic virus*

Varieties	Number of the varieties in the collection of the Inst. Agron.	Number of leaves inoculated	Aver. number of lesions per leaf	Diameter of the lesions (mm.)	Type of lesions
GROUP I.—Strong reactions					
1. Thousand-to-one	21	4	173	0.5	⊙ ·
2. Scotia	....	30	147	0.5–1.5	⊙
3. Fosco	124	20	140	0.6–1.0	⊙ ●
4. Preto	43	22	124	0.4–1.0	⊙ ●
5. Preto S. Paulo	....	22	101	0.4–0.8	⊙ ●
6. Branco	72	10	93	0.5–0.8	⊙ ●
7. Robust	253	24	73	0.5–1.0	⊙ ●
8. Bico de Ouro	36	24	66	0.5–0.8	⊙ ● ·
GROUP II.—Medium reactions					
9. Mulatinho	117	14	203	0.4–1.0	⊙ ● ·
10. Jaboticabal C	30	8	181	0.5	● ·
11. Campineiro	42	10	172	0.5	● ·
12. Idesso	27	16	92	0.4–0.6	● ·
13. Porto Alegre	34	8	73	0.4–0.5	●
14. Porto Alegre	62	16	52	0.5	● ·
15. Bico de Ouro	242	10	52	0.2–0.4	● ·
16. Preto R. Grande	....	20	50	0.3–0.5	● ·
17. Bico de Ouro	66	20	43	0.5–0.8	⊙ ● ·
18. Mulatinho barrado	38	14	42	0.3	●
19. Mulatinho	79	10	26	0.3–0.4	● ·
20. Lambe-beiço	10	16	....	0.5	●
21. Leite	....	......	....	....	●
GROUP III.—Weak reactions					
22. Canna verde	48	16	....	0.3	●
23. Cabreúva	23	16	....	0.2	·
24. Blacos o Lechillas	234	2	224	0.1	·
25. Mulatinho	90	8	137	0.3	● ·
26. Jaboticabal A	28	10	136	0.2–0.4	·
27. Porto Alegre	32	8	114	0.3	·
28. Chita	122	4	106	0.1	·
29. Campineiro	77	6	83	0.2	·
30. Enxofre	126	18	76	0.2	·
31. Mulatinho rajado	3	6	74	0.2	·
32. Pardinho	45	22	72	0.3	● ·
33. Roxo	91	10	63	0.1–0.2	·
34. Mulatinho tupy	109	12	60	0.3	·
35. Chita	121	14	59	0.2	·
36. Ahumados del Coyol	241	6	55	0.2	·
37. Mulatinho commum	4	14	53	0.1	·
38. Rosa	60	8	47	0.1	·
39. Mulatinho claro	39	10	44	....	·
40. Mulatinho alemão	2	8	44	0.1	·
41. Mulatinho	119	14	39	0.1	·
42. Jamaiqueño	212	4	33	0.1	·
43. Higuerilla Blancos	221	4	29	0.2	·
44. Chumbinho	....	4	26	....	·
45. Preto fosco	113	10	26	0.2	·
46. Rapé	256	10	24	....	·
47. Mulatinho novo	1	6	18	....	·
48. Victoria	106	8	16	....	·
49. Bico de Ouro	65	10	11	....	·
50. Mulatinho	37	10	10	....	·
51. Triumpho	26	10	6	....	·
52. Minguinho	58	6	4	....	·
53. Cabreúva	50	6	3	....	·

TABLE 1.—(*Concluded*)

Varieties	Number of the varieties in the collection of the Inst. Agron.	Number of leaves inoculated	Aver. number of lesions per leaf	Diameter of the lesions (mm.)	Type of lesions
GROUP III.—(*continued*)					
54. Porto Alegre	19	8	2	....	.
55. Caeté	63	10	1	....	.
56. Idaho Refugee	252	16	....	....	.
57. Byinton	18	16	....	....	.
GROUP IV.—Negative reactions					
58. Staleys Brown Beauty	203	14	0		
59. Canadian Wonder	204	10	0		
60. Long Fellow	20	14	0		
61. Great Northern	249	12	0		
62. Stringless Greenpod	199	10	0		
63. Baalbek	107	16	0		
64. Riñon Rojo	246	16	0		
65. Nhambú	69	16	0		
66. Mulatinho	73	16	0		
67. Manteiga	9	26	0		
68. Manteiga	41	22	0		
69. Manteiga	40	20	0		
70. Manteigão	40	22	0		
71. Enxofre	111	10	0		
72. Enxofre	53	6	0		
73. Abundancia	14	4	0		
74. Fava de alta classe	15	2	0		

As for the taxonomic position of the bean varieties used in our experiments, this is difficult to determine with exactness because of the marked differences of the system of classification admitted in the descriptions of the genus *Phaseolus* given by Engler-Prantl (4), Bailey (1), Hegi (5) and Bondar (2). There is no doubt however, that all the varieties employed belong to the species *P. vulgaris* L. and, following the classification proposed by Hegi, which is based on the size of the plants—mostly to the subspecies "nanus." d'Utra (3), who published a study about Brazilian bean varieties, subdivided them into various groups, according to shape and color of the seeds.

In conformity with this author, the commercial varieties "enxofre" belong to the group *Phaseolus vulgaris sphaericus;* those named "chumbinho," to the group *Phaseolus vulgaris ellipticus;* the various commercial varieties, commonly named "mulatinho," were included in the group *Phaseolus vulgaris compressus.* The commercial varieties, called "preto" do not yet constitute, according to d'Utra, a uniform botanical group, but have to be included partly in the group *Phaseolus vulgaris compressus,* like the "mulatinho" beans, partly to the group *Phaseolus vulgaris oblongus* and, finally, mostly to the group *Phaseolus vulgaris sphaericus.*

With reference to the foreign commercial varieties of beans, which belong to the collection of the Instituto Agronomico, we can make no statements about their taxonomic position. It seems, however, that most of these varieties belong to the subspecies *"nanus."*

METHOD

The method of inoculation used in the experiments herein reported consisted in putting a drop of the inoculum on the bean leaf to be inoculated and in gently rubbing with the thumb moistened in this liquid the entire upper surface of the leaf. The drops were placed with a pipette and contained always a definite quantity of liquid, in general 0.03 cc. One or two plants of the same varieties, tested with the inoculum, were left as controls and were inoculated with the sap of healthy plants of *Nicotiana tabacum.*

As for the sap containing mosaic virus, all the inoculations were first executed with concentrated mosaic-virus sap, prepared in a sterilized porcelain mortar by adding 1 cc. water per gram of the infected leaves of tobacco. Later, other plants of those varieties of greater interest were treated with the following dilutions: 1:5, 1:10, 1:100 and 1:1000. The number of the plants used in each experiment depended on the available numbers of beans: in general, we made use of eight and never less than two plants.

VARIETAL SUSCEPTIBILITY

In the varieties here studied the lesions begin to develop, as described by

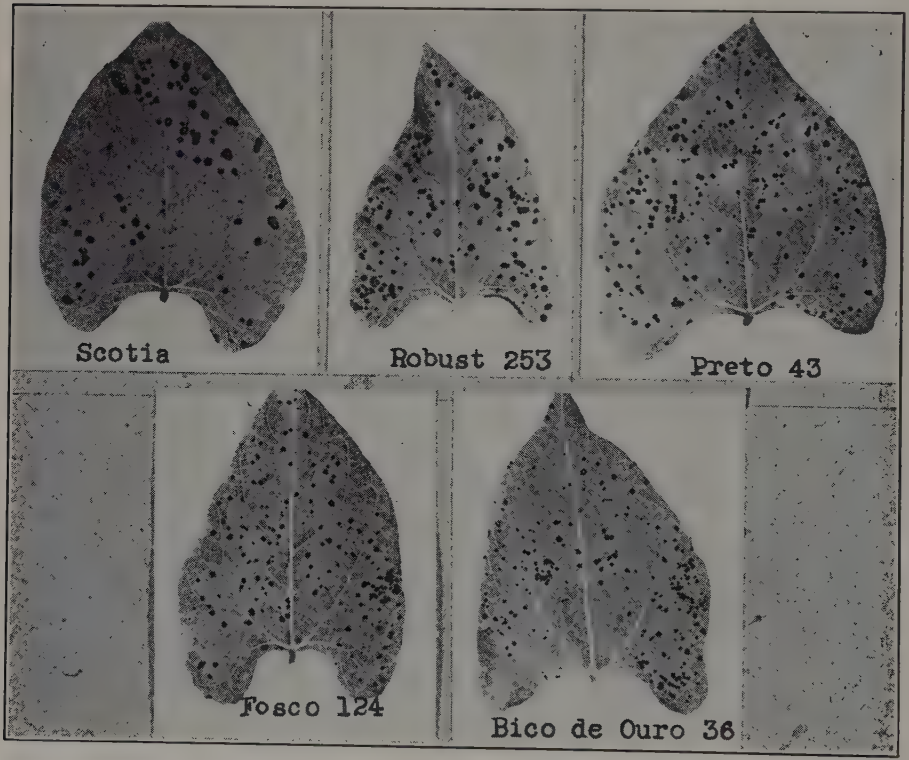

FIG. 1. Primary leaves of bean varieties, inoculated with tobacco-mosaic virus, which exhibit "strong reactions."

Price (6), within 2 to 5 days. Generally, they were observed 5 or more days after inoculation, thus making it possible to observe the number, size, and type of resultant lesions. In this respect our studies differed from those of Price (6), who considered only the number of lesions that appeared on the inoculated leaves.

Price (6) separated the varieties into 3 groups. He considered as belonging to the first group all varieties of which every inoculated plant de-

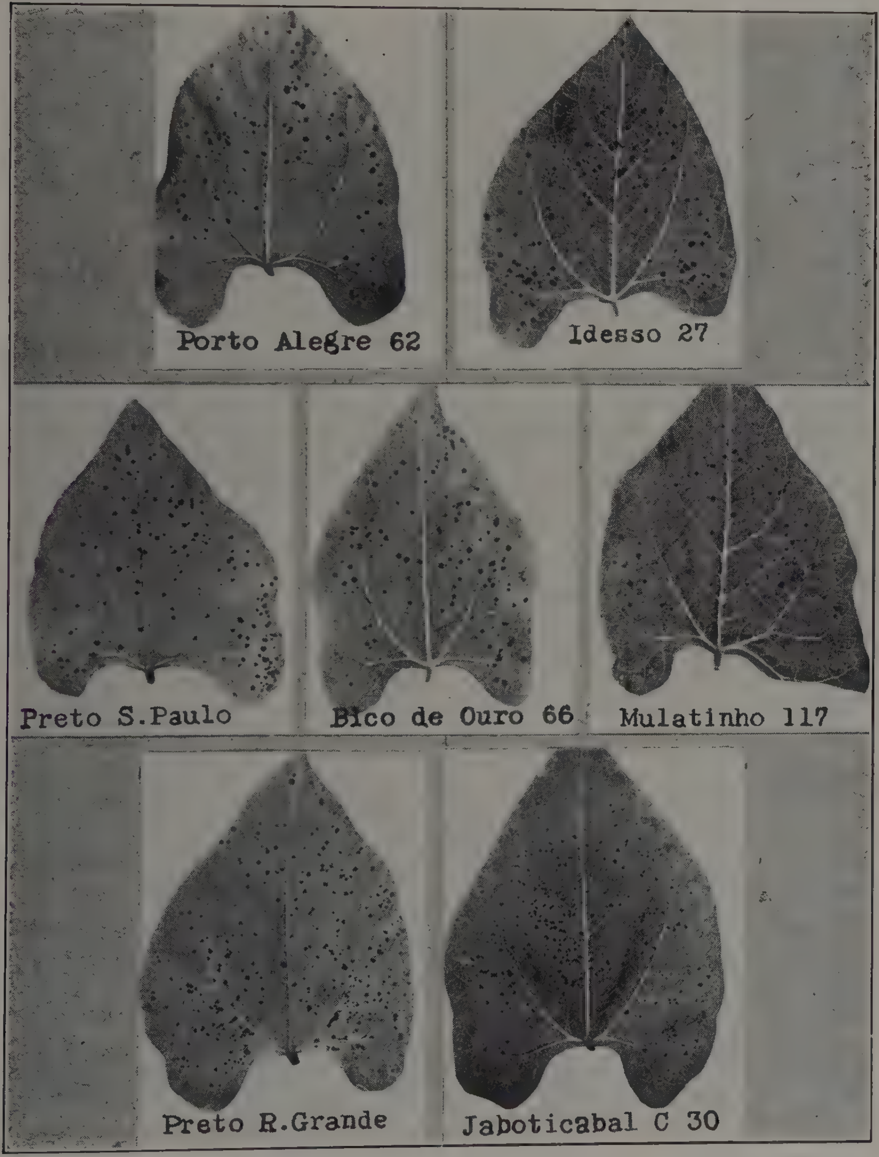
Porto Alegre 62
Idesso 27
Preto S.Paulo
Bico de Ouro 66
Mulatinho 117
Preto R.Grande
Jaboticabal C 30

FIG. 2. Primary leaves of bean varieties, inoculated with tobacco-mosaic virus, which exhibit "medium reactions."

veloped local necrotic lesions. In his second group, lesions appeared on one or more, but not on all, of the inoculated plants. In the third group, none of the inoculated plants developed lesions.

We, however, have found such sharp differences in the visibility of the lesions of the different varieties, that we must consider also the ensemble

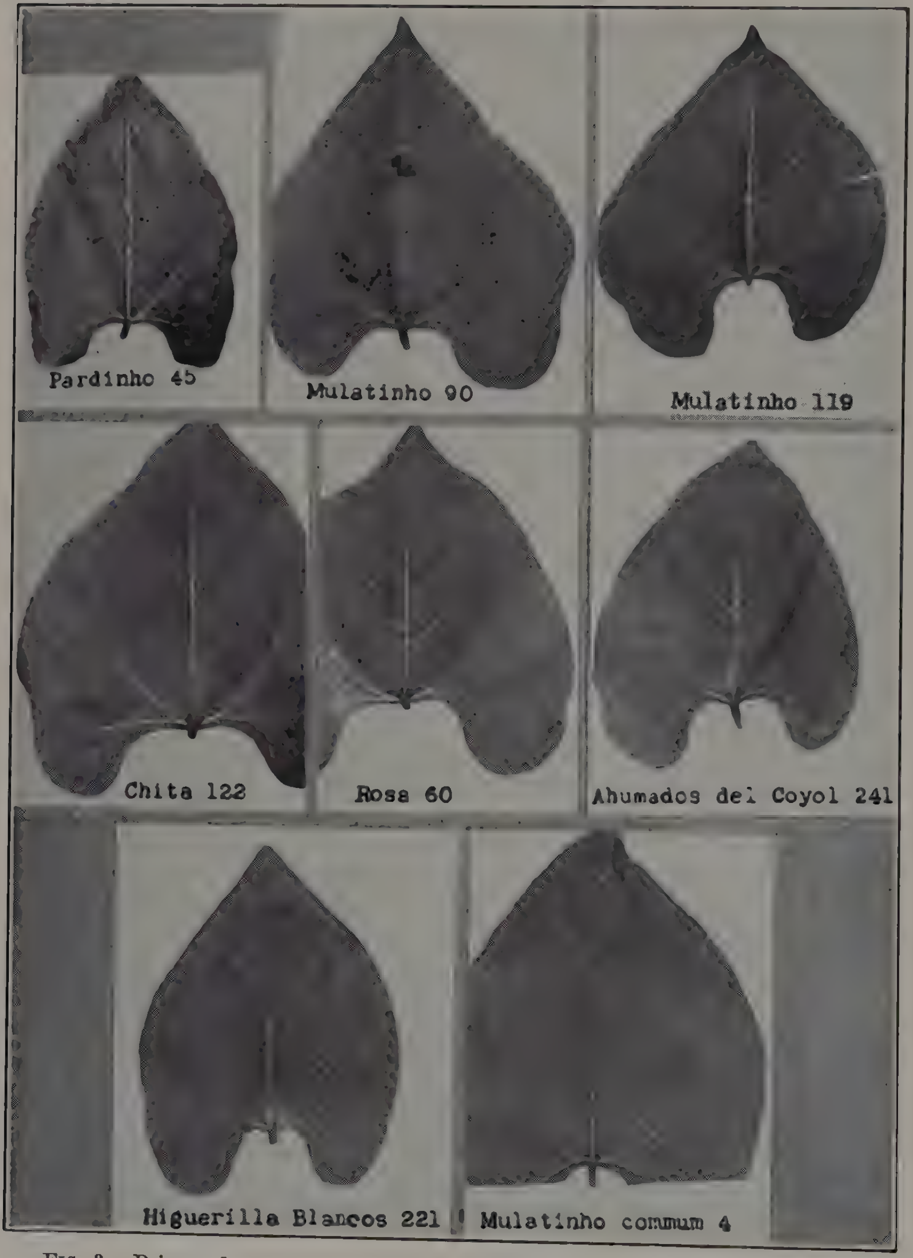
Pardinho 45
Mulatinho 90
Mulatinho 119
Chita 122
Rosa 60
Ahumados del Coyol 241
Higuerilla Blancos 221
Mulatinho commum 4

FIG. 3. Primary leaves of bean varieties, inoculated with tobacco-mosaic virus, which exhibit "weak reactions."

of size, shape, and coloration of the lesions, as very important. On the basis, therefore, of number, size, and type of the lesions, we tried to separate the varieties inoculated into four groups, in conformity with their practical value to the study of tobacco mosaic. These groups are named, respectively, groups of strong, medium, weak, and negative reactions.

Thus, we consider strong-reaction varieties to be those presenting conspicuous, well-marked, individual, and large, local necrotic lesions of ± 1 mm. diameter. These lesions consist in a characteristic brown ring including a lighter nucleus (⊙) (Fig. 1). The medium-reaction varieties show, in the majority of cases, small necrotic lesions, which resemble small, compact, dark-brown plates sufficiently distinct to be readily counted (●) (Fig. 2). The weak-reaction varieties are characterized by very small necrotic lesions, which appear either in great or in small number. These lesions are difficult to count, because of their small size. For this reason the varieties on which they occur are unsatisfactory for use in statistical studies (·) (Fig. 3). The fourth or negative-reaction varieties of beans are characterized by the absence of any local necrotic lesions within 20 days, a period long enough to produce symptoms on the samples of the other groups.

It must be mentioned here, that we inoculated in all the series a set of test plants with water or with sap of healthy tobacco plants. None of these plants developed symptoms of mosaic. The mean results of these experiments, performed with these varieties, are presented in table 1.

DISCUSSION

As explained in the foregoing, all the varieties of beans considered in our study of their importance as indicators of the tobacco-mosaic virus were grouped in four different classes, according to the visibility of the symptomatic necroses produced on the primary leaves after inoculating them with tobacco-mosaic virus, and also according to the decreasing order of the average number of lesions per leaf.

Since our classification was based on more complex characters than were employed by Price, we cannot expect our four classes to coincide strictly with Price's three groups. As a matter of fact, we find in 3 of our 4 classes varieties that develop a great number of local lesions, and that Price probably would have placed in the first group. For practical purposes, however, not all the varieties that show a great number of lesions on the primary leaves have the same value as virus indicators. A rapid counting of the lesions may be performed only on varieties having not only *many* but also *large* lesions. It would seem to us, therefore, that we are, because of *visibility* of necrosis, perfectly justified in including in different groups varieties not distinguishable with reference solely to the *number* of lesions produced on the primary leaves inoculated.

Although we first adopted this system of classification for practical reasons only, we are now convinced that it is more in accord with the real differences of mosaic-virus resistance of the bean varieties studied than is the grouping followed by Price (6). Whereas Price's classification is partly based on a character more or less casual, that is, if a few or all of the inoculated plants develop local symptoms, our classification is founded on varietal qualities based strictly on "resistance" and "immunity."

To support this opinion, we shall here try to explain in what manner differences of reaction in the different bean varieties may arise. In varieties "hypersensitive" to the mosaic virus, the invaded cells are killed soon after infection. In such cases large numbers of small lesions are observed in the inoculated leaves.

In other less sensitive varieties the tissue is killed only after a period long enough to permit invasion of a larger number of cells. Here we find the production of distinctly larger necrotic spots.

There still exists a third case, one in which the varieties exhibit a complete lack of susceptibility to the virus of tobacco mosaic. These varieties are placed in group 4. Here the virus particles do not enter the host cells, hence they do not increase to an extent sufficient to produce pathological effects.

We intend to continue our tests on this subject and to study more exhaustively the physiological qualities connected with the differences of the various bean varieties against mosaic virus.

There derives, from the study of table 1, the question whether we can establish a relation between strength of reaction of the different varieties against mosaic-virus and their taxonomic position.

Even a rapid examination of the varieties belonging to every one of our groups shows no very strict relationship between resistance of the varieties to the mosaic virus and the form and color of the seeds, representative characters essential to taxonomic classification. Thus belong to the first of our groups, distinguished by "strong reactions," varieties with black, white, or brown, flat, round, or oblong, seeds. Considering, however, the distribution of the different systemic unities amongst the 4 groups of our table, we may verify that frequently various varieties of a taxonomic unity belong to the same group shown in table 1. For instance, the 3 varieties of the type "manteiga" and the 2 varieties of the type "enxofre" studied by us, showed no reactions against mosaic virus, belonging thus to the 4th group (Table 1). The majority of our commercial varieties of the type "mulatinho," which is included in our studies, presented medium or weak reactions, whereas the varieties of the type "preto" exhibited strong, or medium reactions. It is, however, true that the facts presented indicate only slightly the existence of a certain relationship between the reaction of the varieties and their taxonomic position.

But, considering that our studies included a relatively small number of varieties of the various types and that the taxonomic positions, not even of our types, are absolutely sure, it is probable that the continuance of this research on a larger scale will disclose the existence of interesting relationships between the taxonomic position and their resistance against mosaic virus of the commercial bean varieties.

SUMMARY

The primary leaves of bean plants of 73 varieties were inoculated with sap containing the tobacco-mosaic virus. The necrotic symptoms observed on the inoculated leaves were used to separate the varieties into 4 groups.

Such separation depends on the number, size, type, and visibility of the necrotic lesions, in which group every variety will be included, and its position within every group will be determined.

In the discussion we try to explain why we have not adopted for our purposes Price's classification, a classification exclusively based upon the character of the number of necrotic lesions.

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LITERATURE CITED

1. BAILEY, L. H. The Standard Cyclopedia of Horticulture **3**: 2574–2577. 1927.
2. BONDAR, G. Feijões cultivados no Brasil. Bol. Secret. Agricult. (Baía). 83 p. 1930.
3. D'UTRA, GUSTAVO. Cultura e conservação dos feijões. Bol. Secret. Agricult. Estado de S. Paulo **4**: 151–179. 1904.
4. ENGLER, A., and K. PRANTL. Die natürlichen Pflanzenfamilien. **3**: (3) 379–380. 1894.
5. HEGI, G. Illustrierte Flora von Mitteleuropa. **4**: (3). Verlag Lehmann (Munich).
6. PRICE, W. C. Local lesions of bean leaves inoculated with tobacco-mosaic virus. Amer. Jour. Bot. **17**: 694–702. 1930.
7. SILBERSCHMIDT, K., and M. KRAMER. Contribuição para o conhecimento do Mosaico do fumo e dos seus hospedeiros selvagens no Brasil. Archiv. Inst. Biol. S. Paulo **9**: 1–20. 1938.

BOTRYTIS AND SCLEROTINIA AS POTATO TUBER PATHOGENS[1]

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(Accepted for publication October 1, 1940)

Although species of *Botrytis* and *Sclerotinia* are more or less common to most vegetable-growing regions, there are a few crops that usually are not affected by these fungi. The potato is one of the outstanding rarely affected vegetables. Under cool, humid conditions *Botrytis cinerea* Pers. sometimes causes a blight of potato plants, and *Sclerotinia sclerotiorum* (Lib.) deBy. causes a stem rot and wilt but neither of these organisms seems to cause a tuber rot that is apparent at digging time. If such decay ever occurs it has been overlooked or at least not recognized as a distinct disease.

In 1927, Folsom[2] reported a rot of stored potatoes caused by *Botrytis cinerea*, and further described and illustrated this disease in 1933 (4), following an investigation of an unusual storage rot of Maine potatoes characterized by a withering of the stem end and a semi-watery brown decay of the internal tissues. When the tubers were almost entirely decayed, the fungus frequently came to the surface through the eyes and there produced the typical grayish-brown spores. In each case the fungus apparently had followed down the stolon from the affected vine and entered the tubers through the stem end.

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[1] An investigation conducted by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Botany Department of the University of Chicago.

[2] U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rptr. **11**: 54. 1927. (Mimeographed.)

The occurrence of *Botrytis* rot (gray mold rot) of potatoes on the market was first noted in January, 1938, in two car lots of Bliss Triumph potatoes received on the Chicago market from California.[3] These potatoes were said to have been raised in the mountains east of Bakersfield at an elevation of about 4500 feet. The crop was harvested about mid-August and shipped to San Francisco, where the potatoes were held in cold storage until late December and then shipped to a nearby city where washing facilities were available. There they were washed, graded, and packed for shipment. On arrival in Chicago they showed defects ranging from 12 to 35 per cent, averaging approximately 22 per cent, consisting principally of bruises and mechanical injury. All crates of potatoes examined showed some botrytis rot. A brown, watery decay, which characterizes the advanced stage of this disease, affected an average of about 5 per cent of the tubers (Fig. 1, A, B, C). In addition to this decay, many crates showed an average of 9 per cent of the tubers affected with a dry type of botrytis rot (Fig. 1, D) appearing as sunken, pitted, and discolored areas, which penetrated into the flesh $\frac{1}{8}$–$\frac{1}{4}$ inch. An inspection of this same stock in a store 2 days later showed an average of 35 per cent of the tubers affected with botrytis rot. In advanced stages of decay the fungus covered a large part of the surface of the tubers and the characteristic grayish brown spores were evident (Fig. 1, A, C). On some almost completely decayed tubers, a few black sclerotia had formed in the appressed surface mycelium. Isolations were made from several tubers showing both the dry and the wet types of decay and in each instance pure cultures of a *Botrytis* were recovered that correspond to the description usually given for *Botrytis cinerea* Pers.

Folsom (4) showed that *Botrytis cinerea* is pathogenic to potato tubers inoculated through wounds and held in high humidity at moderately low temperature. The writer observed that, under somewhat similar conditions, the strain of *Botrytis* isolated from the California potatoes would also cause an extensive decay of other potato tubers. For test inoculations usually 24 midsize tubers were selected for freedom from disease and blemishes and then treated for 30 minutes in a 1 : 240 solution of formaldehyde. After sterilization they were rinsed in distilled water and allowed to dry in a sterile chamber before they were inoculated. Preparatory to inoculation, quarter-inch holes about $\frac{3}{8}$ in. deep were made with a cork borer. The plug was lifted out and a small amount of mycelium from a week-old culture of *Botrytis* grown on potato-dextrose agar was introduced into the opening and the plug was replaced. Usually 10 inoculated tubers and two controls

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[3] Ramsey, G. B. Fruit and vegetable diseases on the Chicago market in 1938. U. S. Dept. Agr. Bur. Plant Ind., Pl. Dis. Rptr. Sup. 114, p. 34, 1939.

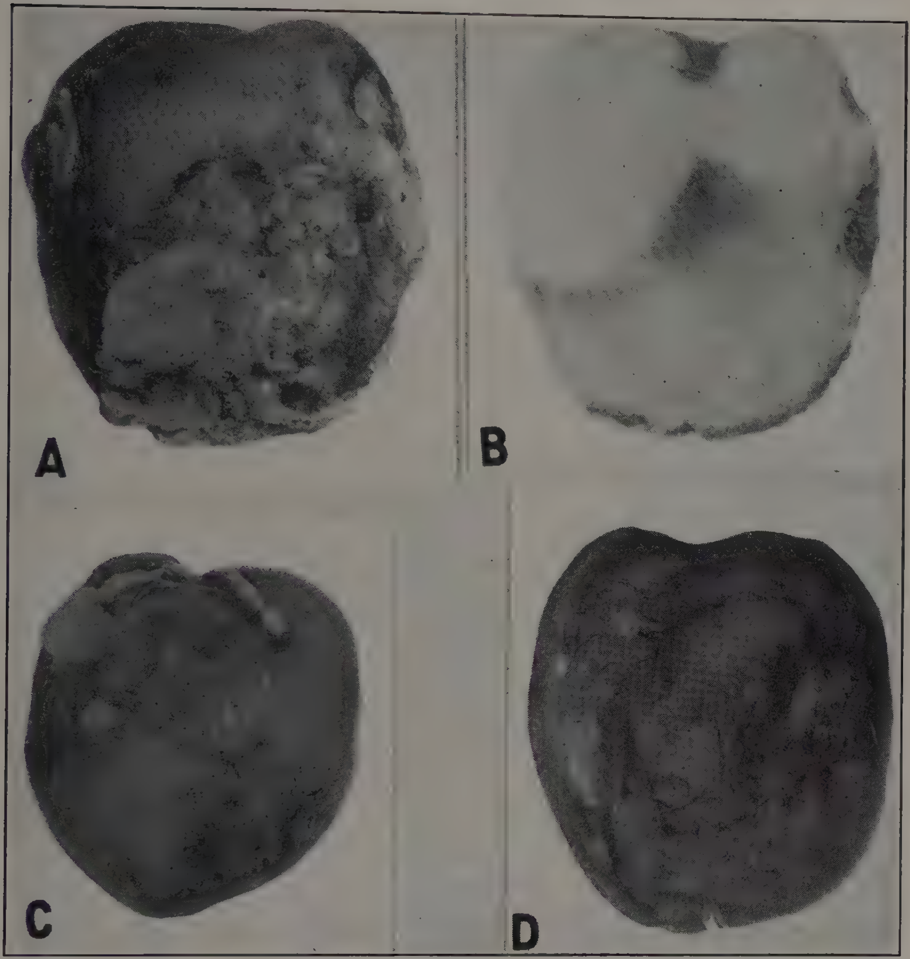

FIG. 1. A. Bliss Triumph tuber, affected with botrytis rot, showing mycelium and spores that had developed during transit. B. Section through tuber A showing the extensive development of brown, semi-watery decay. C. Wet type of botrytis rot showing wrinkling of skin and growth of mycelium and spores through breaks in the skin. D. Dry type of botrytis rot showing sunken spots and areas and scanty development of mycelium.

were placed in a moist chamber containing a small dish of water to insure high humidity. One set of inoculations of this kind was held at room temperature averaging near 70° F., and a similar set was placed under refrigeration at 40° F. Two or more tubers of each set were cut at weekly intervals to check on the development of internal decay. Unless otherwise specified the following notes on the development of decay represent a summary of the results obtained from many experiments.

Many inoculation tests were made in which slightly immature and well-matured Irish Cobbler and Bliss Triumph potatoes were used. Under conditions favorable for infection by *Botrytis,* tubers of whatever stage of maturity seemed equally subject to decay. Of the inoculations made at

70° F., less than 5 per cent induced infection. The resultant rot was brown, moderately firm, and resembled some of the *Botrytis* lesions of the dry type found in the cars of California potatoes. In the few instances when decay occurred at 70°, it seldom extended deeper than $\frac{1}{4}$ to $\frac{1}{2}$ in. in a month.

In contrast to the inoculation experiments conducted at 70° F., most of those tried at 40° gave positive results. Often there was little external evidence of tuber infection at the end of a week, but cut specimens showed decay $\frac{1}{16}$ to $\frac{1}{8}$ in. deep in that time. After 2 weeks many lesions were $\frac{1}{2}$ in. deep and approximately the same diameter on the surface. By the end of a month many lesions were about 1 in. in diameter and $\frac{3}{4}$ to 1 in. deep. The decay was light-brown, moderately soft and watery, like the wet type of Botrytis rot noted in the California potatoes.

To test the possibility of wound infection of potato tubers by *Botrytis* spores, 24 Bliss Triumph tubers were sterilized and rinsed as described above and then shaken together with a rough block of wood in a sterile container until all were bruised and scuffed. Eight were then removed for controls, and the remaining 16 were sprayed with *Botrytis* spores suspended in sterile water. Eight of these and 4 controls were held in a moist chamber at 70° F. The remaining 8 inoculated tubers and 4 controls were kept in a moist chamber in the refrigerator at 40° F. Considerable grayish-brown surface mycelium and some spores developed on the most severely injured areas of the tubers held at 70°, but no decay of internal tissues was observed within a month. The lot held at 40° showed a development of white surface mycelium, but no spores. At the end of a month 3 of these tubers showed one or more watery, brown lesions $\frac{1}{2}$ to 1 in. in diameter and about $\frac{3}{4}$ in. deep. Isolations from these lesions yielded pure cultures of *Botrytis cinerea*. One control tuber at 70° developed Fusarium rot and another bacterial soft rot. The remaining controls showed no decay.

Since typical Botrytis rot was easily obtained when inoculated potato tubers were placed immediately at 40° F., it seemed desirable to test the effect of delayed storage upon the development of decay in inoculated stock. Consequently, one set of tubers was inoculated with mycelium in the usual manner and held 3 days at 70°, then stored at 40° F. A similar set was held continuously at 70°. On examination of half of each set after two weeks, it was found that none of those held at 70° showed decay. The tissues injured by inoculation were well suberized, and there was an extensive development of wound periderm underneath. The tubers held at 40° showed a moderately soft decay about $\frac{1}{8}$ in. deep. The cells at the inoculation wound were suberized but only slight traces of wound periderm had developed.

One month after inoculation the remaining half of each set of tubers was cut. Those held at 70° F. showed no decay, but an abundant development of grayish-brown surface mycelium growing on the injured cells at the point of inoculation. Those held at 40° showed a slight amount of white surface mycelium about the point of inoculation and a light-brown, watery decay penetrating ¾ in. into the tubers. This experiment indicates that at 70° there is not enough wound cork formed within 3 days to prevent infection by *Botrytis* and subsequent decay, provided the temperature and humidity are favorable for the fungus and unfavorable for wound-periderm formation.

Artschwager (1) and others have shown that wound-periderm formation in potato tubers is greatly influenced by temperature and humidity. Under conditions of high humidity, the rate of suberization and wound-cork formation is determined by temperature. In several tests with inoculated potatoes held at 40 and 70° F., the control tubers were examined microscopically to determine the amount of suberization and wound-cork formation at the wounds. Wounded tubers, held in high humidity at 70°, showed suberization of the surface cells and wound-periderm formation within 3 days, whereas those held at 40° F. showed only slight suberization and no development of wound periderm within a month. This no doubt explains for the most part the differences noted in the amount of infection and subsequent decay caused by *Botrytis* under the conditions of the inoculation experiments described. It also indicates that the California potatoes, badly affected with gray-mold rot on the market, probably were harvested under conditions favoring wound infection by *Botrytis,* and that wound-periderm formation was inhibited by the cold-storage temperature to which this stock was subjected shortly after harvesting.

No species of *Sclerotina* have yet been observed to cause tuber decay in field, storage, or market. However, because of the fact that a member of the genus *Sclerotinia* has been shown to be the perfect stage of *Botrytis cinerea* (5) and that botrytis rot has already occurred as an important storage and transit disease of potatoes, it seemed desirable to investigate the possibility of *Sclerotinia* spp. becoming potato-tuber pathogens.

Sclerotinia stem rot and wilt has been found seriously affecting potato plants in Ireland (7), Canada (6), and the United States (3), but in no instance has a tuber rot been reported. Although Eddins (3) reported as high as 70 per cent of the plants killed by *S. sclerotiorum* in some potato fields in Florida, he found no evidence of the tuber decay caused by this organism. Several years ago the writer (10) conducted extensive inoculation experiments with *S. sclerotiorum, S. intermedia* Ramsey, and *S. minor* Jagger. At that time most of the common market vegetables were found to be susceptible to infection by these species. Notable exceptions were the potato tuber, the onion bulb, and in most instances beet roots. At no time was there any evidence of decay of potato tubers inoculated with any of these organisms. This led to the conclusion that the potato tuber is highly resistant to infection. In this connection, however, it is interesting to note that in reporting some inoculation studies with a strain of *Sclerotinia* isolated from sunflowers, Bisby (2) stated that "considerable rot was produced upon potato tubers." He did not describe the decay or state under what conditions the experiments were conducted.

After finding that *Botrytis cinerea,* introduced through wounds, would cause an extensive decay of potato tubers under appropriate moisture and temperature conditions, it was decided to test the pathogenicity of *Sclerotinia* species to potato tubers under the same conditions used in the tests with *Botrytis.*

In the many experiments conducted at 40° F. *Sclerotinia sclerotiorum* induced no infection, and less than 25 per cent of the inoculations caused infection when the tubers were held at 70°. *S. minor* usually was strongly pathogenic to the tubers held at 70°, often showing near 100 per cent infection, whereas the tubers held at 40° rarely showed any decay, although in a few experiments 15 to 25 per cent became infected. In tests with *S. intermedia,* a high percentage of infection was always obtained at 40°. Ordi-

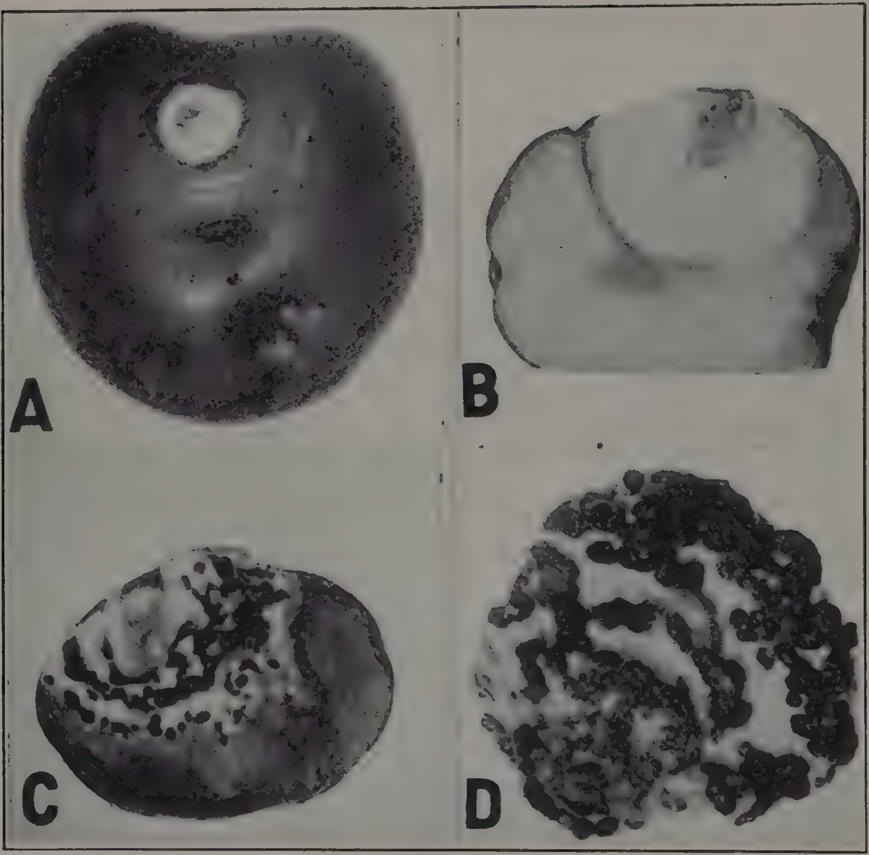

FIG. 2. A. Bliss Triumph tuber inoculated with *Sclerotinia intermedia* and held at 40° F. for one month. Note the bleaching of the skin about the inoculation wound and the slight wrinkling indicating the extent of the decay underneath. B. Section through a tuber similar to A. The white, watery decay is characteristic for *S. intermedia* at 40° F. C. Mycelium and sclerotia of *S. intermedia* developed in a week at 70° F. after a lesion similar to B had developed at 40° F. D. A Bliss Triumph tuber completely decayed by *S. intermedia* within two months at 40° F. Note the extremely large sclerotia that develop at this temperature.

narily, more than 50 per cent of the inoculations resulted in decay at this temperature, whereas at 70° more than 90 per cent of the inoculations produced no decay. In tests with Irish Cobbler, Russet Burbank, and Bliss Triumph potatoes no differences in pathogenicity were noted.

The decay of potatoes produced by each of the *Sclerotinia* species is watery and soft, as in other vegetables. In advanced stages of decay the tubers become leaky under pressure. In all stages the affected tissues are white and appear bleached lighter than the normal tissues of the tuber (Fig. 2, A, B). Even in advanced stages of decay when sclerotia have formed and the whole tuber is involved, only a light brown discoloration is produced, and there is no odor (Fig. 2, C, D).

Many inoculation experiments were conducted at the potato-storage temperature of 40° F., since it seemed likely that if *Sclerotinia* species ever became serious as potato-tuber pathogens, it would be when contaminated tubers are placed in cool storage, as in the case of botrytis tuber rot. Potato tubers inoculated with *Sclerotinia intermedia* and placed immediately at 40° usually showed 50 to 75 per cent decay after a week. On an average the penetration of the tuber issues was about $\frac{1}{8}$ in. in 1 week, $\frac{1}{4}$ in. in 2 weeks, $\frac{1}{2}$ in. in 3 weeks and 1 in. in a month. In the few instances when *S. minor* proved pathogenic at 40°, the depth of penetration did not exceed $\frac{1}{4}$ in. per month. *S. sclerotiorum* never gave positive results in any of the inoculation tests at 40° F.

As a check against the inoculation experiments conducted at 40°, a corresponding number of experiments were tried at 70° F. In these *Sclerotinia minor* proved by far the strongest pathogen. In many experiments the infections ranged from 47 to 100 per cent. A moderately rapid development of decay occurred at 70°, and there was extensive development of mycelium and sclerotia about the point of inoculation within 2 weeks. The characteristic white, watery decay penetrated into the tuber about an inch in two weeks.

Inoculations with *Sclerotinia sclerotiorum* and *S. intermedia* seldom resulted in the production of decay in potato tubers held at 70° F. In most instances less than 10 per cent of them resulted in the disease; several inoculations were fruitless. This indicates that under moderate temperature and humidity conditions neither of these two species is likely to become pathogenetically important to the potato. However, it is possible that under moderate temperature and high humidity at harvest time wounded tubers may become infected with *S. minor* and develop decay during transit and marketing. Moreover, if such infected stock be placed in cool storage, the decay will progress at 40° F. This was shown experimentally by holding tubers at 70° for 2 days following their inoculation with *S. minor* and storage for 2 weeks at 40°. A high percentage of decay was produced under those circumstances. In a few instances inoculations made with *S. intermedia* also resulted in decay under the conditions just mentioned.

Previous investigations (9, 10) have shown that each of the 3 species of

Sclerotinia under consideration can grow continuously at 32° on potato-dextrose agar, and that *Sclerotinia intermedia* develops more rapidly at this temperature than do the other two species. The ability of this species to cause infection and decay of potato tubers at low temperature is shown by the following experiment in which the tubers were inoculated in the usual manner with the 3 species of *Sclerotinia,* and then were placed immediately at 32°. Two weeks later the tubers showed that more than half the inoculations with *S. intermedia* were positive. The typical white, watery decay had developed an average of ¼ in. from the edge of the inoculation wound. Neither *S. sclerotiorum* nor *S. minor* caused any decay at this temperature.

The experimental evidence indicates that quick suberization of cells and development of wound periderm at 70° F. in injured potato tubers usually is an effective barrier to penetration by *Sclerotinia sclerotiorum* and *S. intermedia,* but not by *S. minor.* The differences in pathogenicity noted at 70° are no doubt associated to some extent with the rate at which these fungi grow; but that this is not the complete explanation is indicated by the fact that although *S. sclerotiorum* and *S. minor* grow at approximately the same rate on potato-dextrose agar at 70°, *S. minor* is pathogenic to potato tubers more than 3 times as often as *S. sclerotiorum.* Furthermore, *S. intermedia* causes infection of potatoes held at 70° almost as often as does *S. sclerotiorum,* but on potato-dextrose agar it grows at about half the rate of the latter species at that temperature. In the experiments conducted at 40° in which only slight suberization but no wound-periderm formation was involved, *S. intermedia* was the only species that consistently caused decay. At 40° this species grows almost twice as fast on potato-dextrose agar as either of the other species.

From a practical standpoint it seems probable that *Sclerotinia minor* is the only one of these three species of *Sclerotinia* that might become of economic importance on potatoes harvested and handled under ordinary conditions. Although this fungus is rather widely distributed, it is not so common as *S. sclerotiorum.* The latter species is present in almost all vegetable-growing regions. However, the fact that it has not been reported as a potato tuber-rotting organism, even in fields where most of the plants were severely affected, indicates that it is not strongly pathogenic to the tubers, and all of the experiments reported herein point to the same conclusion.

The distribution of *Sclerotinia intermedia* is not known, but apparently it is limited, and, insofar as can be determined now, the fungus is not likely to become an important potato-tuber pathogen. Furthermore, Eddins (3) has shown that this species appears unable to parasitize potato plants, whereas both *S. sclerotiorum* and *S. minor* cause a severe stalk rot and wilt of growing potato plants.

SUMMARY AND CONCLUSIONS

Botrytis tuber rot (gray-mold rot) has been reported as a storage disease of Maine potatoes following stem-end infections. A similar rot is here reported as a serious market disease observed in a shipment of California potatoes, following infection at wounds.

A *Botrytis* of the *cinerea* type was isolated from many tubers in two carloads of Bliss Triumph potatoes showing a semi-watery, brown, odorless decay. Wound-inoculation experiments show this organism strongly pathogenic to potato tubers held at 40° F. in high humidity. Similar experiments conducted at 70° seldom resulted in the production of decay, thus indicating that suberization and wound-periderm formation usually take place soon enough at 70° to be an effective barrier to invasion by this fungus. However, inoculated tubers held at 70° for 3 days and then stored at 40° developed extensive decay. This shows that not enough wound periderm is formed within 3 days at 70° to prevent infection and subsequent decay, if low temperature inhibits further development of wound periderm.

Freshly wounded tubers sprayed with *Botrytis* spores suspended in sterile water developed the characteristic watery, brown decay when stored at 40° F., but no decay occurred in tubers held at 70°, although there was an abundant growth of mycelium over the most severely injured areas.

Since Groves and Drayton (5) have shown that *Botrytis cinerea* has a *Sclerotinia* stage, it seemed desirable to test whether species of the latter genus are pathogenic to potato tubers. In wound inoculation tests with *Sclerotinia sclerotiorum*, tubers, held at 70° in high humidity, developed decay in comparatively few instances. Similar experiments with *S. intermedia* also usually resulted in few infections, but most tubers inoculated with *S. minor* developed extensive decay within 2 weeks.

In inoculation experiments conducted with potato tubers at 40° F. under high humidity *Sclerotinia sclerotiorum* did not produce rot, and *S. minor* caused rot only rarely, whereas *S. intermedia* usually caused an extensive white, watery soft rot.

Bliss Triumph potatoes inoculated with *Sclerotinia intermedia* and stored immediately at 32° F. developed decay within 2 weeks. Neither *S. sclerotiorum* nor *S. minor* caused decay at that temperature.

Experimental evidence indicates that under the usual harvesting and handling practices *Sclerotinia minor* could become important as a cause of tuber rot. If wounded potatoes contaminated with *S. intermedia* were placed immediately in refrigerated storage or common storage near 40°, this organism also would be able to cause extensive decay. *S. sclerotiorum,* the most common and widely distributed species of *Sclerotinia,* appears least likely to cause potato tuber rot.

A microscopic study of the wounds in control tubers held at 40° F. in high humidity showed that some suberization had taken place but that no wound periderm had formed within a month. Similar wounded control tubers kept at 70° in high humidity showed extensive suberization and wound-periderm formation within 3 days. This suggests that the danger of decay resulting from wound infections by *Botrytis* or *Sclerotinia* species may be reduced greatly by holding potatoes at moderate temperature and humidity for 3 days or more after harvesting.

Under conditions favoring the development of decay, Bliss Triumph and Irish Cobbler tubers were equally susceptible to infection by the species of *Botrytis* and *Sclerotinia* tested.

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LITERATURE CITED

1. ARTSCHWAGER, ERNST. Wound periderm formation in the potato as affected by temperature and humidity. Jour. Agr. Res. [U.S.] **35**: 995–1000. 1927.
2. BISBY, G. R. Stem-rot of sunflower in Manitoba. Sci. Agr. **2**: 58–61. 1921.
3. EDDINS, A. H. Sclerotinia rot of Irish potatoes. Phytopath. **27**: 100–103. 1937.
4. FOLSOM, DONALD. *Botrytis cinerea* as a cause of potato tuber rot. Phytopath. **23**: 993–999. 1933.
5. GROVES, J. W. and F. L. DRAYTON. The perfect stage of *Botrytis cinerea*. Mycologia **31**: 485–489. 1939.
6. LACHAINE, O. W. Sclerotial disease of the potato. Quebec Soc. Protect. Plants Ann. Rept. **14 (1921–22)**: 105–109. 1922.
7. PETHYBRIDGE, GEO. H. Investigations on potato disease. Jour. Dept. Agr. Tech. Instr. Ireland **10**: 241–256. 1910.
8. ————. Investigations on potato diseases. (Seventh report) Journ. Dept. Agr. Tech. Instr. Ireland **16**: 564–596. 1916.
9. RAMSEY, G. B. *Sclerotinia intermedia*, n. sp., a cause of decay of salsify and carrots. Phytopath. **14**: 323–327. 1924.
10. ————. *Sclerotinia* species causing decay of vegetables under transit and market conditions. Jour. Agr. Res. [U.S.] **31**: 597–632. 1925.

A PHOTOELECTRIC METHOD AND ITS USE FOR DETERMINATION OF FUNGUS GROWTH RATES[1]

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In the course of physiological studies it frequently becomes necessary to use colony growth as a criterion of the effects of experimental conditions or a particular medium. The most widely accepted method is the gravimetric determination of the net increase in synthesized dry matter. The method is slow and laborious, the accuracy depending upon the sensitivity of the balance used and on the care with which the necessary manipulations, such as drying and weighing, are carried out. The method is, in general, applicable to cultures in liquid media in which many fungi do not grow well.

Growth also may be estimated by means of measurements made with the micrometer and the mechanical stage (5). This method is slow, and its accuracy depends largely upon the skill of the operator. Usually, an entire hyphal system cannot be followed and the tips requiring measurements are at different angles with the plane of the microscope stage, resulting in errors of measurement, corrections for which are impracticable. The individual hyphae measured must be assumed to be representative and the growth of the colony considered similar.

By the substitution of a photoelectric system many of these disadvantages can be eliminated and others greatly reduced. The colony can be considered as a whole, taking into account growth in 3 dimensions in a given period of time. The number of determinations possible in a day's time can be greatly increased. Agar media, as well as broth, can be used.

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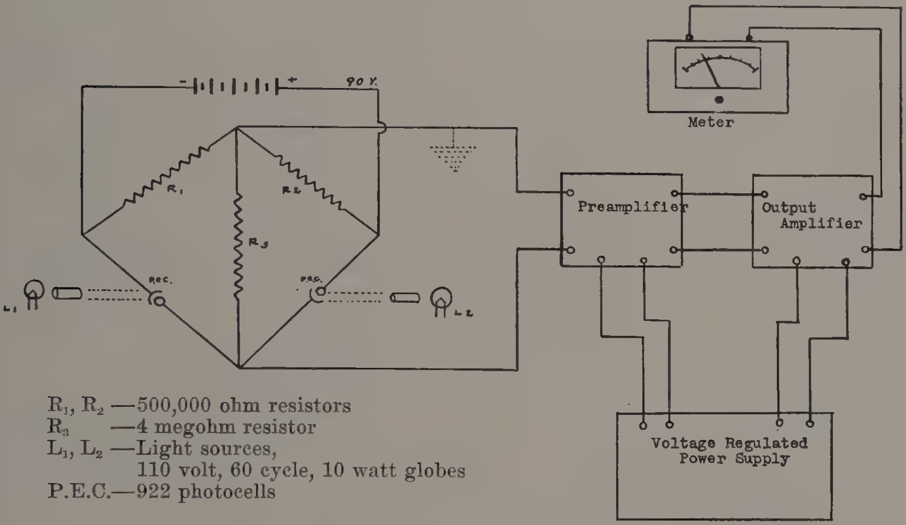
[1] Contribution from The Clayton Foundation for Research.

In the photoelectric method it is desirable to have a reference standard to which the unknown colony can be compared. In order to obtain this, and at the same time establish electrical stability of the instrument (3), a balanced double-cell system has been used, one being employed as a constant reference cell and the other acting to compare the unknown against this standard.



R_1, R_2 —500,000 ohm resistors
R_3 —4 megohm resistor
L_1, L_2 —Light sources,
110 volt, 60 cycle, 10 watt globes
P.E.C.—922 photocells

FIG. 1. Block diagram of instrument, showing functional units.

As shown in the circuit diagram (Fig. 1), the 2 photocells have been substituted in 2 legs of a wheatstone bridge and a 4-megohm load resistance inserted in the place of the conventional galvanometer (2 and 6). A slight unbalance of the bridge will cause a relatively high voltage drop to appear across this 4-megohm resistor, this voltage being proportional to the amount of unbalance existing in the bridge. Variation in intensity of the source of light, due to any external factors, is rendered negligible by the fact that the system is maintained in an essentially balanced condition with the lamps on the same circuit and subject to same fluctuations in current.

The voltage drop produced across the load resistor is applied as a bias to the grid of the preamplifier tube. This tube is directly coupled into the grid of an output tube in a conventional D.C. amplifier circuit. A 0–10 milliampere meter is placed in the plate circuit of the output tube and the readings obtained in this manner reflect the condition existing in the measuring bridge (1). By the use of a 2-stage D.C. amplifier in conjunction with the bridge-circuit input, it is possible to adjust the constants of the circuit to expand or compress the scale of readings wherever desired, giving the greatest accuracy within the regions of most interest and relegating the remainder to a small portion of the entire meter scale. In this instance it was found desirable to include a selective shunt across the meter coil in order that large colonies might be read easily and at the same time protect the meter from prolonged overloads. While the shunt is in the circuit the readings of the meter are multiplied by the factor 2.

The entire instrument is operated from a conventional regulated power supply consisting of a voltage doubler, filter, and voltage-regulator tube.

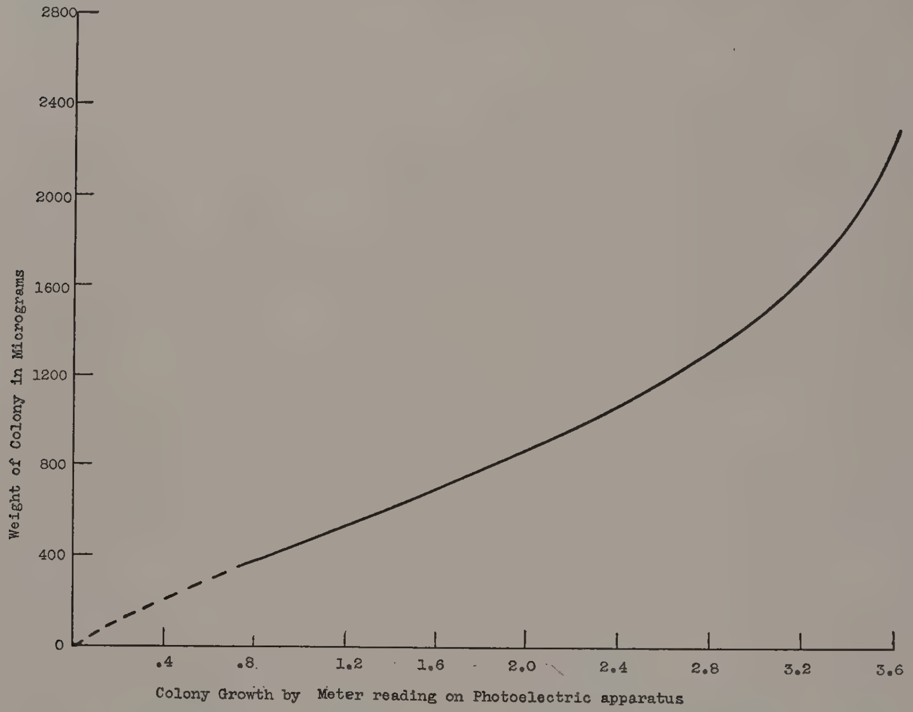


FIG. 2. Relation between weight of fungus colony and photoelectric-meter reading.

Figure 2 is a calibration chart showing the close relation between the meter readings and the actual dry weight of the fungus colony. An error of approximately plus or minus 2 per cent should be expected within the range used, which is of small significance in the determination of resistance if the wide range of growth encountered is taken into consideration. It might be further noted at this point that the variation in weight to produce this error is in the order of a few micrograms, in itself beyond the accuracy of the average gravimetric determination.

In the course of work carried on in the University of Texas laboratory it became necessary to measure the susceptibility and resistance of individual cotton plants to the root-rot organism, *Phymatotrichum omnivorum* (4). For comparable values the growth of the fungus under standard conditions and on standard root-decoction media was employed. Since the project required the growth determinations on over 20,000 samples per year, none of the usual methods was practical, and this special photoelectric comparator was designed, constructed, and standardized. The instrument has been used with entire satisfaction for the past 3 years, readings indicating the difference between 2 entire colonies or between inoculum and the colonies developing from such inocula. The readings correct for the color of the medium and for the fungus added originally as inoculum.

In the present study liquid media were used, standard inocula being made from liquid cultures. The method is as follows: The mature root system was washed, dried, and ground to pass a 20-mesh screen. The medium contained 2 per cent dried ground root in 1 per cent dextrose. Extraction is accomplished by steeping in the autoclave for 20 minutes at 15 lb. steam pressure. The medium is filtered, divided, and inoculated with standard inocula of the root-rot organism. Since, with the cotton root-rot organism, no viable spores are available, disks cut from a uniform mat of vegetative hyphae by means of a sterile paper punch furnish the inoculation material. Controls of 1 per cent dextrose are included in each day's run. After incubation at 30° C. (which is near optimum temperature for this fungus) for 72 hours the colonies produced are carefully removed, placed on a slide, blotted to remove excess moisture, and placed under the unknown side of the instrument. The amount of growth is then indicated on the meter. The readings for a given root are averaged and divided by the average reading secured for the controls for that particular day. The empirical figure obtained is a means to show proportionality and represents growth rate acceleration or retardation. The amount of growth of the root-rot fungus in the presence of the water-soluble constituents of an individual cotton root is taken as a criterion of the relative resistance or susceptibilty of that plant to the attack of the fungus under field conditions. Growth acceleration is assumed to be proportional to susceptibility to the disease; retardation of growth in turn indicates resistance. These figures form a basis for selection of plants showing the greatest degree of resistance and are those chosen for subsequent propagation. Selfed seeds of these selected individuals are used for plantings.

CONCLUSIONS

The photoelectric method used for determining the amount of growth produced by the root-rot fungus under standard conditions is an accurate and speedy method. We average 400 observations or more a day, or at the rate of 150 observations per hour.

The readings secured by this method correlate closely with gravimetric methods for larger colonies; for smaller colonies the photoelectric method surpasses in accuracy.

This method reduces to a minimum the variable factors met with in other techniques.

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LITERATURE CITED

1. Application Notes and Characteristics of Type 922 Phototube. Radio Corporation of America. Sept. 1937.
2. BORDEN and BEHAR. Bridges and their applications. Instruments 7: 233–234. 1934.
3. FINLAY, ROBERT. Photometering raw silk. Electronics' Engineering Manual, 59–62. 1938.
4. GOLDSMITH, G. W., and ELIZABETH JANE MOORE. Field tests of the resistance of cotton to *Phymatotrichum omnivorum.* Phytopath. 31: 452–463. 1940.
5. MOORE, ELIZABETH JANE. Growth relations in culture of the cotton root-rot organism, *Phymatotrichum omnivorum.* Phytopath. 23: 525–537. 1933.
6. SMYTHE and MICHELS. Advanced electrical measurements. D. Van Nostrand Co. (New York). 1932.

FIELD TESTS OF THE RESISTANCE OF COTTON TO PHYMATOTRICHUM OMNIVORUM[1]

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INTRODUCTION

Resistance to bacterial and fungus diseases is well known to constitute a genetic character in many instances. From the work on wheat rusts it is obvious that in this case resistance is Mendelian (1 and 3), but that various physiological strains of rusts, varieties of host plants, and environmental conditions greatly complicate the picture.

Studies on the Phymatotrichum root rot of cotton have shown that all varieties of cotton are susceptible in the field if conditions are favorable for the disease, but have given no evidence to indicate that the fungus occurs in physiologic strains with different degrees of virulence or aggressiveness. A wide variety of susceptible hosts is known, while the monocots are immune under ordinary field conditions (9). Resistance seems attributable to water-soluble thermostable materials present in the roots in varying amounts in plants not susceptible to the disease (4, 6, and 7).

It would seem probable that low degrees of resistance, if present in cottons, would be difficult to detect in the field, because of the great variability in attack shown by the root-rot fungus. It would seem probable also, since the various species of cotton have survived in a wild or semi-wild state in the same general region in which the root rot is endemic, that some degree of resistance has arisen, at least in this group.

Several workers have noted the differences in growth obtained with pathogens growing in extracts of roots of susceptible varieties as opposed to the reduced growth maintained by the pathogen on extracts from immune varieties (5 and 8). It has been found in this laboratory that standard cotton-root decoctions are suitable in different degrees for the growth of the root-rot fungus under standard and constant conditions (2). Decoction media made from immune plants retard or fail to support growth of *Phymatotrichum,* while similar decoction media made from susceptible species support abundant growth. Since no completely resistant varieties of cotton are known, we have no breeding stock by the use of which immune and desirable strains can be developed by the usual methods. If some degree of resistance exists in cotton and can be recognized, progress may be expected by the method of selection and selfing because of the frequent cross pollination occurring in cotton.

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[1] The Clayton Foundation for Research.

METHODS

1938. In the summer of 1936, variety plats were established near Austin in which all plants were selfed and selections for subsequent plantings were based on individual growth-rate tests of the root-rot fungus under standard conditions on root decoctions of similar concentrations. Selections were made in this way in 1936 and 1937 and the resulting selfed seed was planted in 1938 in an area of Houston black-clay soil that had shown approximately 80 per cent kill from root rot for the two preceding years.

The test plot was planted in randomized plats totalling 3,000 row feet, and included selections of some 28 varieties. Treatment was identical with that of the adjoining farm field; no fertilizer was applied, little or no thinning was carried out, and 3 cultivations were made. Plants were counted at 10-day intervals and all dead plants cut off at the soil surface in order to insure maximum infestation and avoid errors in counting. As a control 4,000 plants taken in areas selected at random on opposite sides of the test plot in the adjoining field of Kasch cotton were used.

During the season all flowers were selfed, and the selfed seed was harvested at the end of the season, along with the root systems of all surviving plants. These root systems furnished the material for individual growth-rate tests, the results of which furnished data for selections of individuals to be planted the following season.

RESULTS

1938. The results are given in table 1 in terms of percentage kill. For obvious reasons these results are greatly condensed (Footnote, Table 1). Only the final kill was recorded for the control. During the season the total kill in the unselected Kasch cotton was 72 per cent, while that for all the selected progeny was 57.5 per cent. Thus, 2 years of selections, based on laboratory growth-rate tests alone, resulted in a reduction in kill of 24.5 per cent. Considering the results of all selected individuals as given in table 1, the final kill at the end of the season has a standard-deviation value of 18.4 per cent. When we compare this with the reduction figure of 24.5 per cent the result seems significant.

Comparison of the varieties under selection with the nonselected Kasch (Table 1) shows a marked improvement in some of them; a thing to be expected if we assume that some varieties contain no resistance in the genetic complex, while others do. The most promising varieties, results of which are statistically significant, are: Cluster Durango-Wacona, Texas Special, Acala, Harper, Native Hopi, Barberton, Stoneville, and Dixie Triumph. These are obviously the varieties which should be subjected to further selection, breeding, and field tests.

TABLE 1.—*Summary of kill in root-rot area in 1938 (Kimbro, Texas)*

[Original number plants { 5043 selected / 4000 nonselected]

Varietal name selected	No. of selections	No. of replications	Original No. July 10	Percentage kill on July 21	July 31	Aug. 10	Aug. 20	Aug. 30	Sept. 9	Sept. 19
Kekchi[a]	11	24	364	35.7	41.5	56.0	67.3	70.6	71.4	71.7
Tuxtla-Trice	1	3	40	15.0	25.0	30.0	45.0	55.0	55.0	55.0
Trice	5	5	107	21.5	29.9	38.3	45.7	56.0	58.8	58.8
Acala-Trice	1	1	18	11.0	27.5	55.0	82.5	82.5	82.5	82.5
Acala	5	7	1231	13.9	16.3	26.5	33.8	37.6	39.1	39.4
Lonestar	4	5	262	31.7	36.6	49.2	65.6	68.3	70.9	70.9
Cook Wilt-resistant	2	5	132	43.2	59.8	75.7	90.1	93.2	93.2	93.2
Rowden	1	3	79	27.8	31.6	48.1	55.7	55.7	56.9	58.1
Coker	6	8	159	27.0	39.6	59.1	63.5	65.4	65.4	65.4
Stoneville	1	3	88	31.8	31.8	32.9	40.9	48.8	50.0	50.0
Dixie-Triumph	4	12	238	23.9	28.0	41.6	49.1	49.6	50.4	50.4
370–390–01	3	9	294	25.8	32.0	47.9	58.1	61.9	65.0	65.0
Delfos	3	7	148	33.8	41.8	55.4	70.3	72.3	72.3	72.3
Delta & Pineland	3	3	310	11.3	16.4	28.4	41.3	43.9	46.1	46.1
Indian Green	1	3	102	38.2	40.2	45.1	51.0	52.9	58.9	60.9
Sunshine	2	4	106	35.8	39.6	46.2	61.3	63.2	67.9	72.6
Kasch	1	1	72	26.4	31.9	36.1	52.7	59.7	61.1	62.5
Ferguson-Triumph	4	9	197	36.5	48.7	57.8	70.5	72.1	73.1	73.1
Cluster Durango-Wacona	1	1	15	0.0	0.0	20.0	20.0	20.0	20.0	20.0
Lankart	1	2	37	54.0	78.4	100.0	100.0	100.0	100.0	100.0
Mebane	3	9	179	35.2	44.7	60.9	75.4	80.4	80.4	81.0
Texas Special	1	1	25	8.0	8.0	16.0	28.0	28.0	36.0	36.0
Cliett	3	4	101	15.0	25.0	46.0	56.0	63.0	67.0	71.0
Bagley	3	6	104	32.7	59.6	71.1	89.4	93.3	97.1	97.1
Harpers	1	3	47	29.8	29.8	40.4	48.9	48.9	48.9	48.9
Sudan	3	7	112	16.9	21.4	43.7	54.5	57.1	57.1	58.0
Native Hopi	3	5	128	15.6	20.3	28.9	38.3	38.3	45.3	46.0
Barberton	7	11	344	13.7	23.5	28.5	40.1	41.0	41.9	41.9
Weighted means				23.9	29.7	41.8	52.3	55.3	57.0	57.5
Standard deviation										18.4
Unselected Kasch			4000							72.0

TABLE 1.—(*Continued*)

Kekchi strains	No. of selections	No. of replications	Original No. July 10	Number dead on July 21	July 31	Aug. 10	Aug. 20	Aug. 30	Sept. 9	Sept. 19
3	2	3	45	20	25	34	38	38	38	38
4	2	4	66	25	28	40	45	52	53	53
7	1	4	33	7	7	8	12	13	15	16
15	1	1	39	......	......	5	12	15	15	15
17	2	4	65	19	22	30	39	40	40	40
27	1	2	24	16	18	22	22	22	22	22
28	1	3	38	26	26	32	36	36	36	36
37	1	3	54	17	25	33	41	41	41	41
Totals	11	24	364	130	151	204	245	257	260	261
Percentage kill, weighted means[a]				35.7	41.5	56	67.3	70.6	71.4	71.7

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[a] The weighted mean at a given date is derived by dividing the total number of plants dead at that time by the original total under consideration.

TABLE 2.—*Summary of kill in root-rot areas in 1939 (Kimbro, Texas)*
Selected selfed varieties (Total No. plants 5468)

Varietal name	No. of selections	No. of replications	Original No. June 10	Percentage kill on									
				June	July				August			September	
				20	1	10	20	29	8	18	28	7	16
Kekchi[a]	24	63	501	5.2	9.2	12.8	17.7	22.4	26.9	35.7	40.5	46.7	47.3
Trice	11	36	221	4.1	10.9	15.4	17.6	20.8	22.6	30.3	33.9	36.7	37.6
Lonestar	9	34	274	3.6	9.1	12.0	16.4	18.6	23.0	31.4	34.3	42.0	43.4
Cook wilt-resistant	9	20	166	5.4	8.4	9.6	10.8	13.3	18.1	23.5	29.5	30.7	35.5
Acala	17	42	407	3.4	7.6	11.5	14.5	20.6	24.6	32.2	40.5	45.9	48.9
Acala x-rayed	4	11	113	0.0	3.5	9.7	10.6	13.3	14.2	24.8	31.9	39.8	43.4
Dixie-Triumph	14	53	473	4.7	7.4	11.0	13.1	19.2	23.5	29.4	34.9	38.9	40.0
Stoneville	3	15	140	7.1	9.3	15.7	22.1	27.1	32.9	39.3	40.7	43.6	46.4
Rowden	5	14	118	6.8	10.2	13.6	20.3	26.3	32.2	42.4	52.5	55.9	57.6
Half-and-Half	5	15	155	8.4	13.5	16.8	18.7	23.2	25.8	32.3	33.5	37.4	40.0
Coker	4	8	76	7.9	11.8	11.8	14.5	23.7	25.0	32.9	35.5	35.5	38.2
370–930–01	11	32	330	4.5	7.0	11.2	13.6	19.1	23.0	30.3	33.4	37.3	39.4
Delfos	9	29	233	6.9	13.7	20.2	22.7	31.3	36.5	46.8	51.5	58.8	60.5
Delta & Pineland	9	19	153	5.9	13.1	16.3	21.6	29.4	36.6	43.1	46.4	47.1	47.7
Cleve-Wilt	1	2	17	0.0	23.5	35.3	41.2	52.9	52.9	52.9	64.7	76.5	76.5
Virescent Yellow-leaf	7	9	89	10.1	13.5	16.9	16.9	24.7	29.2	39.3	48.3	50.6	51.7
Indian Green	5	10	88	3.4	5.7	5.7	8.0	8.0	8.0	15.9	25.0	29.5	29.5
Harper	4	13	194	1.5	3.6	5.2	6.7	9.8	13.9	20.1	25.3	32.5	34.0
Sunshine	3	9	66	3.0	6.1	15.1	16.7	18.2	22.7	30.3	34.8	42.4	47.0
Cliett	5	12	85	5.9	14.1	17.6	20.0	29.4	35.3	43.5	44.7	49.4	49.4
Kasch	1	3	16	0.0	6.3	18.7	25.0	31.2	43.7	62.5	62.5	68.7	68.7
Texas Special	1	6	73	13.7	19.2	27.4	31.5	38.4	46.6	53.4	56.2	57.5	57.5
Ferguson Triumph	15	41	400	3.3	6.5	8.2	11.7	17.5	22.7	35.0	43.7	49.5	52.0
Cluster Durango-Wacona	3	5	39	0.0	2.6	2.6	5.1	5.1	5.1	12.8	25.6	43.6	43.6
Lankart	1	1	9	0.0	11.1	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
Qualla	2	3	20	10.0	10.0	10.0	10.0	10.0	10.0	15.0	25.0	25.0	30.0
Hurley Special	1	3	27	0.0	0.0	3.7	3.7	7.4	11.1	18.5	25.9	25.9	25.9
Carlsbad	1	3	31	0.0	6.5	22.6	35.5	45.2	48.4	51.6	67.7	67.7	67.7
Mebane	2	7	63	1.6	3.2	7.9	11.1	20.6	23.8	28.6	36.5	39.7	41.7
Shan Khaki	1	2	29	10.3	10.3	13.8	13.8	20.7	20.7	31.0	41.4	69.0	69.0
N. A. (*G. herbaceum*)	4	13	145	7.6	10.3	11.7	14.5	17.2	22.1	29.7	35.2	40.0	42.1
B. 30.1	1	1	7	14.2	14.2	14.2	14.2	14.2	14.2	14.2	28.4	28.4	28.4
Uganda	1	2	12	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
Sudan	7	31	346	1.2	3.8	6.9	9.0	13.8	16.2	21.7	27.5	34.1	34.9
Barberton	7	23	167	3.6	7.8	9.6	12.0	16.8	20.9	28.7	35.3	36.5	37.1
Native Hopi	1	3	20	0.0	0.0	5.0	5.0	5.0	10.0	10.0	10.0	10.0	10.0
Weighted means				4.7	8.6	12.2	12.6	19.5	23.1	32.5	39.0	43.2	44.9
Standard deviation													17.01

TABLE 2.—(*Continued*)

Kekchi strains	No. of selections	No. of replications	Original No. June 10	Number dead on									
				June	July				August			September	
				20	1	10	20	29	8	18	28	7	16
2	2	7	20					1	1	4	6	12	13
3	2	6	43	4	5	5	6	6	7	9	10	10	10
4	3	9	77	3	9	14	17	26	29	33	42	42	43
7	3	8	92	7	10	11	14	16	17	26	28	35	36
11	2	5	42	2	3	3	4	6	9	16	18	21	21
15	5	10	77	1	8	12	19	24	30	36	40	45	45
17	4	7	59	3	3	4	6	10	11	19	20	23	23
27	1	2	16	4	5	6	7	7	7	7	7	7	7
35	1	3	18	1	1	2	2	2	3	4	5	7	7
37	1	6	57	1	2	7	9	14	21	25	27	32	32
Total	24	63	501	26	46	64	84	112	135	179	203	234	237
Percentage kill, weighted means				5.2	9.2	12.8	17.7	22.4	26.9	35.7	40.5	46.7	47.3

TABLE 3.—*Summary of kill in root-rot areas in 1939 (Kimbro, Texas) F_2 generation of 3349 selected hybrids*

Varietal name	No. of selections	No. of replications	Original No. June 10	Percentage kill on									
				June	July				August			September	
				20	1	10	20	29	8	18	28	7	16
Kekchi × Kekchi	2	2	25	8.0	16.0	16.0	16.0	24.0	24.0	32.0	36.0	36.0	36.0
Kekchi × Cook	3	8	78	5.1	11.5	16.7	20.5	26.9	32.1	41.0	44.9	46.2	46.2
Kekchi × Red Spot Upland	3	5	54	7.4	9.3	13.0	14.8	16.7	27.8	29.6	31.5	35.2	35.2
Kekchi × Trice	2	8	71	1.4	8.5	14.1	16.9	16.9	23.9	31.0	33.8	40.8	47.9
Trice × Trice	12	27	243	4.1	6.2	8.6	10.7	14.8	17.3	23.5	29.6	34.9	36.2
Trice × Dixie Triumph	3	6	71	7.0	9.9	19.7	21.1	26.8	29.6	40.8	45.1	47.9	49.3
Tuxtla × Trice	5	7	56	1.8	8.9	14.3	17.9	19.6	19.6	26.8	32.1	35.7	35.7
Acala × Trice	4	14	150	6.0	9.3	10.7	14.7	18.7	23.4	26.7	30.0	38.0	40.7
Acala-Trice × 370–930–01	1	3	26	0.0	0.0	0.0	0.0	7.7	11.5	23.1	27.0	50.0	53.8
Lonestar × Cook	8	12	116	6.0	7.8	12.9	14.7	19.0	22.4	26.7	29.3	31.0	34.5
Lonestar × Arkansas 17	1	6	34	0.0	2.9	5.9	8.8	11.8	14.7	26.5	35.3	44.1	44.1
Lonestar × Half-and-Half	3	11	95	3.2	4.2	5.3	8.4	15.8	21.1	32.6	41.1	47.4	48.4
Lonestar × Clus. Dur.–Wacona	1	1	12	8.3	16.7	16.7	16.7	16.7	16.7	16.7	16.7	41.7	41.7
Lonestar × Acala	2	2	16	6.2	6.2	12.5	18.8	18.8	25.0	25.0	25.0	25.0	25.0
Lonestar × Delfos	3	4	54	0.0	5.6	12.9	14.8	14.8	22.2	37.0	42.6	46.3	46.3
Lonestar × Tuxtla	1	5	59	1.7	5.1	5.1	6.8	20.3	23.7	40.7	52.5	59.3	61.0
Cook × Cook	15	22	273	5.9	8.4	11.0	15.0	19.0	25.6	32.2	34.8	37.0	39.8
Cook × N.A.	1	4	35	2.9	2.9	2.9	2.9	2.9	5.7	11.7	17.1	20.0	20.0
Cook × Cliett	3	5	30	3.3	10.0	16.7	16.7	20.0	33.3	40.0	40.0	43.3	43.3
Cook × Harpers	3	7	56	0.0	0.0	3.6	5.4	17.9	23.2	28.6	28.6	28.6	28.6
Cook × Acala	2	2	12	0.0	0.0	16.7	25.0	33.3	33.3	41.7	41.7	41.7	41.7
Cook × Delfos	2	3	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8	9.5	19.0
Cook × Red Spot Upland	1	3	26	7.7	15.4	23.1	23.1	27.0	27.0	27.0	27.0	27.0	27.0
Cook × Survival A–10	1	2	13	0.0	0.0	0.0	0.0	7.7	7.7	15.4	15.4	15.4	15.4
Cook × Texas Special	1	5	38	5.3	10.5	15.8	23.7	28.9	31.6	34.2	44.7	50.0	52.6
Cook × Bagley	2	6	50	4.0	4.0	6.0	10.0	20.0	22.0	40.0	56.0	58.0	58.0
Acala × 370–930–01	3	5	74	9.5	12.2	18.9	24.3	31.1	33.8	37.8	40.5	41.9	44.6
Acala × Dixie Triumph	3	4	29	3.4	6.9	10.3	10.3	13.8	13.8	13.8	13.8	13.8	13.8
Acala × New Boykin	3	6	77	6.5	10.4	15.6	19.5	24.7	28.6	46.8	54.5	59.7	62.3
Acala × Delta and Pl.	2	9	67	3.0	6.0	10.4	11.9	11.9	16.4	20.9	23.9	37.3	47.8
Acala × Sunshine	2	4	37	0.0	0.0	5.4	5.4	5.4	5.4	8.1	13.5	18.9	27.0
Acala × Half-and-Half	1	1	11	0.0	9.1	18.2	18.2	27.3	45.4	45.4	45.4	54.5	54.5
Acala × Kasch	2	2	13	15.4	46.2	53.8	69.2	69.2	76.9	76.9	76.9	84.6	84.6
Acala × Stoneville	2	5	47	8.5	19.1	25.5	27.7	29.8	31.9	40.4	44.7	51.1	51.1
Acala × Hurley Special	1	2	11	0.0	18.2	63.6	63.6	63.6	63.6	63.6	63.6	63.6	63.6
Acala × Acala-Trice	2	4	38	13.2	15.8	15.8	18.4	23.7	28.9	34.2	39.5	42.1	42.1

TABLE 3—(*Continued*)

Varietal name	No. of selections	No. of replications	Original No. June 10	Percentage kill on									
				June	July				August			September	
				20	1	10	20	29	8	18	28	7	16
Acala × Native Hopi	2	3	36	0.0	5.6	13.9	22.2	25.0	27.8	27.8	27.8	27.8	27.8
Acala × Delfos	4	6	93	21.5	25.8	30.1	40.9	45.2	48.4	52.7	52.7	55.9	57.0
Acala × Acala	6	18	153	4.6	11.1	15.0	15.7	24.8	32.0	39.9	39.9	45.1	47.1
Acala × Vir. Yellow-leaf	2	4	32	6.2	15.6	15.6	18.8	18.8	18.8	25.0	34.4	40.6	40.6
Acala × Kekchi	2	4	25	4.0	20.0	20.0	28.0	36.0	40.0	40.0	40.0	44.0	44.0
Dixie Triumph × Dixie Tri.	4	12	97	5.2	10.3	15.5	18.6	29.9	36.1	43.3	47.4	51.5	57.7
Dixie Triumph × Kasch	3	8	51	7.8	7.8	9.8	17.6	19.6	23.5	29.4	37.3	41.2	41.2
Dixie Tri. × D. & Pl.	3	7	63	3.2	7.9	15.9	15.9	22.2	27.0	34.9	44.9	44.9	46.0
Rowden × Indian Brown	2	4	40	17.5	20.0	20.0	25.0	27.5	27.5	30.0	37.5	42.5	45.0
Arkansas 17 × Acala	1	2	21	4.7	9.5	19.0	19.0	23.8	28.6	47.6	66.7	71.4	76.2
Arkansas 17 × Lonestar	1	2	18	5.6	5.6	5.6	5.6	11.1	11.1	27.8	27.8	27.8	27.8
370–930–01 × Native Hopi	1	2	16	0.0	6.3	6.3	6.3	12.5	31.2	37.5	43.8	43.8	43.8
370–930–01 × D. & Pl.	2	7	72	4.2	4.2	4.2	6.9	11.1	16.7	27.8	31.9	33.3	34.7
370–930–01 × New Boykin	3	10	79	3.8	13.8	17.7	30.4	43.0	51.9	60.8	62.0	62.0	62.0
370–930–01 × Express	2	9	86	4.7	4.7	7.0	7.0	8.1	10.5	17.4	20.9	29.1	29.1
Delfos × Karakhaddi	1	3	25	4.0	4.0	4.0	8.0	8.0	8.0	16.0	16.0	16.0	16.0
Delfos × Kekchi	4	6	57	1.8	12.3	15.8	17.5	24.6	28.1	40.3	50.9	57.9	59.6
Delfos × Rowden	1	3	27	0.0	3.7	14.8	18.5	29.6	29.6	40.7	51.8	59.3	59.3
Delfos × Arkansas 17	1	3	20	5.0	10.0	10.0	10.0	10.0	15.0	35.0	45.0	55.0	55.0
D. & Pl. × Indian Green	3	5	40	2.5	7.5	7.5	7.5	20.0	22.5	27.5	27.5	30.0	32.5
Vir. Yellow-leaf × Kelly	1	1	2	0.0	0.0	0.0	0.0	0.0	0.0	50.0	50.0	50.0	50.0
Sunshine × 370–930–01	1	1	8	0.0	0.0	0.0	0.0	0.0	0.0	12.5	12.5	12.5	12.5
Sunshine × Red Leaf	1	2	13	0.0	0.0	7.7	7.7	7.7	15.4	46.2	53.8	53.8	53.8
Sunshine × Cleve-Wilt	1	1	9	22.2	22.2	22.2	22.2	22.2	33.3	44.4	44.4	44.4	44.4
Sunshine × Qualla	2	4	35	2.8	2.8	2.8	2.8	5.7	8.6	14.3	37.1	48.6	48.6
New Boykin × New Boykin	1	6	51	9.8	13.7	23.5	25.5	35.3	41.2	56.9	64.7	72.5	74.5
N. Boykin × Clus. Dur.-Wa.	2	3	35	11.4	11.4	11.4	14.3	14.3	20.0	25.7	34.3	40.0	42.9
Ferguson Triumph × Acala	1	1	9	11.1	11.1	22.2	22.2	22.2	22.2	22.2	22.2	22.2	22.2
Clus. Dur.-Wa. × Cliett	2	9	52	9.6	11.5	13.5	19.2	23.1	36.5	53.8	65.4	80.0	80.0
Clus. Dur.-Wa. × Acala	1	3	16	0.0	12.5	18.7	25.0	37.5	43.7	56.2	62.5	68.7	68.7
Clus. Dur.-Wa. × Mebane	1	2	15	6.7	20.0	20.0	20.0	33.3	66.6	93.3	93.3	93.3	93.3
Hurley Special × Mebane	1	4	28	3.6	7.1	7.1	7.1	14.3	14.3	28.6	35.7	53.6	64.3
Mebane × Ferguson Tri.	3	8	52	1.9	5.8	11.5	17.3	21.2	25.0	32.7	40.4	48.1	48.1
Cliett × Ramgamati	1	3	20	10.0	25.0	30.0	30.0	35.0	35.0	40.0	45.0	45.0	45.0
Weighted means				5.2	9.0	12.8	20.3	22.7	27.7	32.9	36.6	41.9	43.6
Standard deviation													16.75

METHODS

1939. The methods used in 1939 were identical with those of 1938, except for the fact that 2 neighboring plots were used giving a total of 10,000 row feet. Both plots were located, as in 1938, in heavily infested areas showing a kill of about 80 per cent for several preceding seasons. Controls were sections of nonselected cotton chosen at random from the farm fields adjoining the test plots and also plats of nonselected varieties carried within the randomized planting.

RESULTS

1939. The condensed results are given in tables 2, 3, and 4. The weighted mean kill for all nonselected plants, including both those in the open field and the varieties included in the randomized plots, is 72.5 per cent (Table 4). The values obtained in the randomized plots and in the adjoining farm field were similar, being 70.5 per cent kill in the first group and 74.5 per cent in the second. In comparison with this the mean for all selected varieties, which in 1939 represented the third selection based on the fungus growth rates, is 46.2 per cent kill. This is a reduction of 26.3 per cent as the apparent result of 3 years selection on the 36 varieties listed in table 2. The standard deviation, 16.8 for the selected varieties, indicates that the reduction of 26.3 per cent below the average percentage kill of nonselected varieties is significant.

If we consider only these 44 varieties tested in 1938 and again in 1939 (Table 2) there appears a value for the total kill 19.33 less in 1939 than in 1938. It would thus appear that the selection of 1938 reduced the percentage kill by 19.33. A similar comparison of the kill of nonselected Kasch in 1938 and 1939 (Tables 1 and 4) shows no significant difference; no apparent progress was made by selection in the Kasch variety. Of the varieties showing minimum values in 1938, 3 reappear in 1939, namely, Native Hopi, Harper, and Barberton. The progress obtained by the 1938 selection, as shown by the difference in the final kill of 1938 and 1939, is highly variable probably because of the small numbers of plants used. Of the 24 varieties tested during both seasons, 19 showed varying progress toward increased resistance and 5 negative results. In this favorable group Hopi seems most promising, in view of the high resistance and response to selection.

In general, the kill in the various replications of a single selection are consistent.

The F_2 hybrids, the results of which are given in table 3, have an average mean kill of 43.6 per cent. There is no significant difference between this value and that obtained for the selfed-variety group (Table 2), 44.9 per cent, since the standard deviation is 2.88. If the values obtained for each selfed variety and for each hybrid progeny are considered individually, it will be seen that the selfed group showed but one result of 20 or more below the mean; that is, 2.8 per cent of the group showed relatively high resistance. In comparison with this the F_2 hybrids showed 7, or 10.8 per cent, of the

TABLE 4.—*Summary of kill in root-rot areas in 1939 (Kimbro, Texas), Unselected varieties*

Varietal name	No. of replications	Original No. June 10	Percentage kill on									
			June	July				August			September	
			20	1	10	20	29	8	18	28	7	16
In test plots:												
Acala	10	99	0.0	0.0	2.0	7.1	15.2	32.3	49.5	69.7	90.9	94.9
Delfos	10	105	0.0	0.9	1.9	4.8	12.4	20.0	31.4	42.9	56.2	67.6
Sudan	15	166	4.2	10.2	16.3	22.3	32.5	37.9	47.6	52.4	55.4	58.4
Total		370	1.9	4.9	8.4	13.2	22.2	31.3	43.5	54.3	65.1	70.5
Farm field adjoining:												
Kasch		3000					24.4		50.0	63.5	68.2	72.2
New Boykin		3000					40.7		64.7	72.8	74.1	76.9
Total		6000					32.5		57.3	68.6	71.0	74.5
Total for all unselected		6370										72.5
Standard deviation												16.3

group with these low values. When it is remembered that the F_2 hybrid generation has been subjected to but one selection based on the fungus growth rate, it is obvious that these hybrids proved to be better material for selection than the selfed varieties. This result might be expected, if we are dealing with the Mendelian characters.

In order to estimate the progress made in a single variety, comparisons may be made between the percentage kill in selected and nonselected representatives of the same variety. In 1939, after 3 selections, the final percentage kill for Acala varieties was: selected 48.9, nonselected 94.9; Delfos, selected 60.5, nonselected 67.6; Stoneville, selected 46.4, nonselected 55.8[2]; Dixie Triumph, selected 40.0, nonselected 44.7[2]; and Sudan, selected 34.9, nonselected 58.4.

The results obtained in the nonselected groups are shown in table 4. The average for varieties used in the randomized plots was 70.5 per cent, and that obtained in the adjoining field areas of nonselected cotton was 74.5 per cent. Since the standard deviation is 15.38 the difference is not significant and it appears that the controls in the field were as satisfactory as those in the randomized plots. It further indicates that the investigation of that particular season was comparable in the two areas.

Figure 1 shows graphically the 2 years' results. The average kill of the

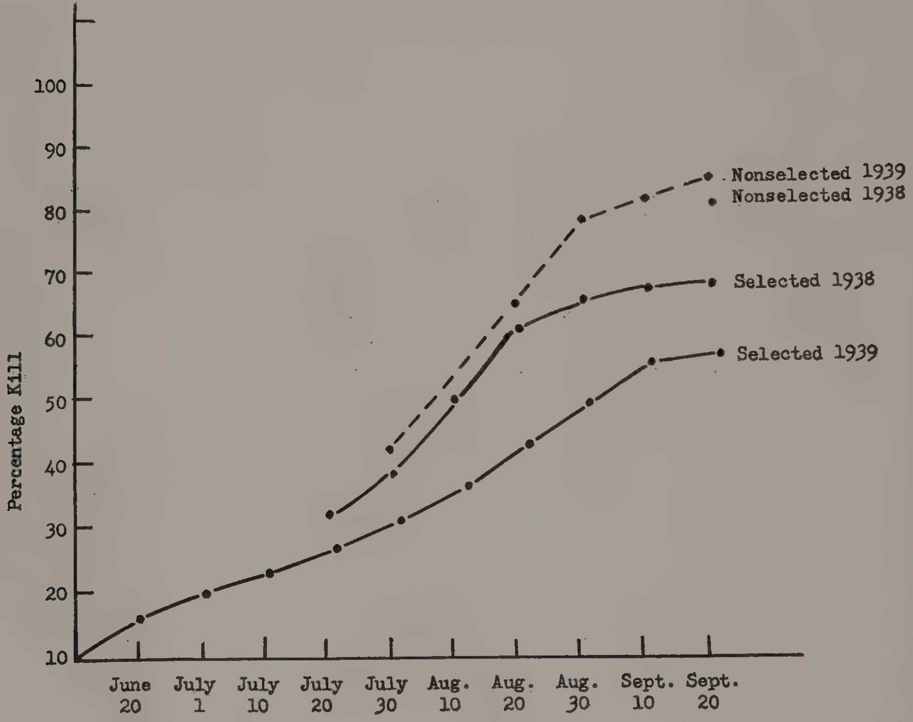

FIG. 1. Average kill from root rot of selected and unselected cotton at Kimbro, Texas, in 1938 and 1939.

[2] Supplied courtesy Dr. L. M. Blank, Dept. Agr., from data obtained simultaneously and in the same field.

nonselected cotton was comparable both years. The average kill in the selected varieties shows a marked improvement from 1938 to 1939, or a reduction in kill of 26.3 per cent.

CONCLUSIONS

By means of selection based on the fungus growth rates, resistance of cotton to root rot has been tested in the field during the past two seasons. Selection has produced a definite increase in resistance. Resistance in some degree exists in many varieties of cotton. If these various degrees of resistance are evaluated by laboratory tests, selection brings about a general average increase in this character; but this effect varies in degree with different varieties, among which Native Hopi and Sudan are most promising. This method of selection and testing in the field produces, in general, maximum results when F_2 hybrids are employed.

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Cotton Root-rot Investigation and Research,
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LITERATURE CITED

1. Aamodt, O. S. The inheritance of growth habit and resistance to stem rust in a cross between two varieties of common wheat. Jour. Agr. Res. [U.S.] **24**: 457–469. 1923.
2. Adair, L. A., and Elizabeth Jane Moore. A photoelectric method and its use for determination of fungus growth rates. Phytopath. **31**: 448–452. 1940.
3. Biffen, R. A. Studies on the inheritance of disease resistance. Jour. Agr. Sci. **2**: 109–128. 1907.
4. Ezekiel, W. N., and J. F. Fudge. Studies on the cause of immunity of monocotyledonous plants to Phymatotrichum root-rot. Jour. Agr. Res. [U. S.] **56**: 773–786. 1938.
5. ———, J. J. Taubenhaus, and J. F. Fudge. Growth of *Phymatotrichum omnivorum* in plant juices as correlated with resistance of plants to root-rot. Phytopath. **22**: 459–474. 1932.
6. Greathouse, Glenn A., and Neil E. Rigler. The chemistry of resistance of plants to Phymatotrichum root-rot. IV. Toxicity of phenolic and related compounds. Am. Jour. Bot. **27**: 99–108. 1940.
7. Moore, Elizabeth J. Growth relations in culture of the cotton root-rot organism, *Phymatotrichum omnivorum*. Phytopath. **23**: 525–537. 1933.
8. Ranker, Emery R. The nature of smut resistance in certain selfed lines of corn as indicated by filtration studies. Jour. Agr. Res. [U. S.] **41**: 613–619. 1930.
9. Taubenhaus, J. J., and W. N. Ezekiel. A rating of plants with reference to their relative resistance and susceptibility to Phymatotrichum root-rot. Tex. Agr. Exp. Stat. Bull. No. 527. 1936.

VEIN CLEARING, A TRANSMISSIBLE DISEASE OF PRUNUS[1]

S. M. ZELLER AND A. W. EVANS[2]

(Accepted for publication October 1, 1940)

A new virosis of *Prunus* has been discovered in Oregon. It occurs widely in this State, and specimens have been received from one location in Washington. This disease was first observed in the summer of 1936.

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[1] Published as Technical Paper No. 357, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

[2] The authors wish to express their appreciation for the kind cooperation of Messrs. Walter Clore and J. D. Menzies of the Irrigation Branch Experiment Station, Prosser, Washington. They have furnished diseased scion-wood used in some of the grafting experiments, and also sent pressed leaves and invaluable field notes.

It affects in particular several varieties of cherries. Very similar symptoms are found on Italian prune. Among the ornamental flowering cherries, like symptoms have been found on 6 varieties of *Prunus serrulata,* while varieties of *P. yedoensis, P. sieboldii,* and *P. subhirtella* do not show symptoms. J. A. Milbrath first discovered the disease on flowering cherry, particularly in the Kwanzan variety.

Among orchard cherries the disease is confined to black varieties of sweet cherry (*Prunus avium*). It affects Bing, Black Republican, and Lambert varieties.

Orchard cherry trees in which the disease is not very far advanced, make nearly a normal growth. Trees in which infection is of several years' standing show considerable dwarfing. Those, apparently recently infected, may show only slight symptoms of the disease. Such symptoms usually will be confined to a few diseased spurs or to one branch. The disease is expressed through rather definite symptoms of foliage, branches, and fruit.

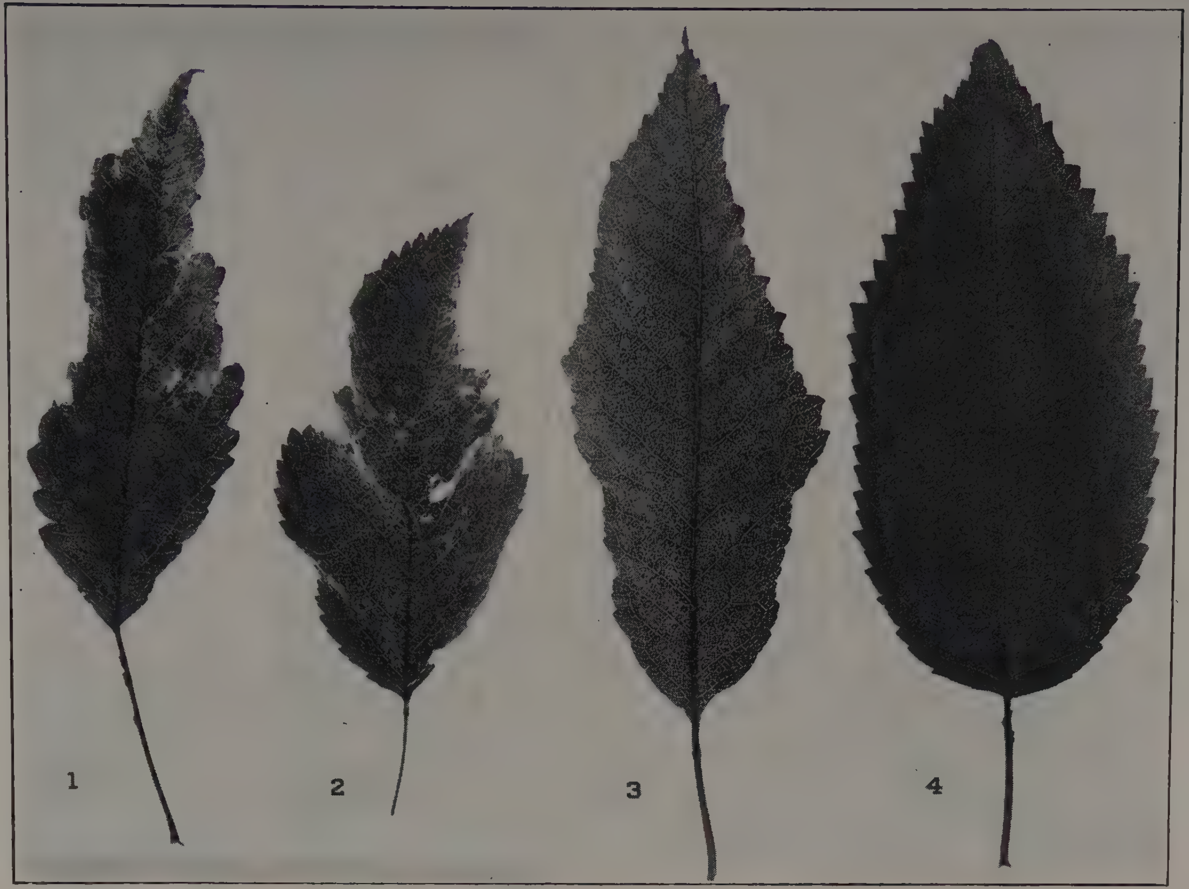
LEAF SYMPTOMS

The first apparent symptoms are in the leaves. The veins are cleared throughout a leaf or only in localized areas. The leaf pattern is extremely variable and always accompanied by vein-clearing. In most varieties of cherries the margins of the leaf are made irregular, usually the deeper indentations occurring adjacent to the more concentrated vein-clearing. (Pl. I.) At times whole portions of the leaf are absent, either on the margins or within the leaf blades. In the latter case two conditions arise. In one condition the mesophyll is greatly lacking and, conversely, the cleared veins are concentrated. In the other the leaf tissues are wanting, sometimes as a marginal indentation extending nearly to the midvein of the leaf and bounded above and below by main lateral pinnate veins, or sometimes, within the blade of the leaf as elongate, elliptic, or slot-like perforations. All of these conditions usually result in narrower than normal leaves.

Enations in the form of small blistered proliferations along the under side of the main veins are sometimes found. This feature was observed as an early current-season symptom.

Whereas vein clearing in the leaves is the chief symptom noticeable by transmitted light, there is a characteristic silvering of the upper surface shown by reflected light. The silvering of the surface is more intense over the areas of concentrated veins showing clearing. This might be evidence of the liberation of pectases in these areas or, perchance, the middle lamellae did not form under the pathological conditions existing at these foci of intensified symptoms.

By midsummer the leaves of diseased trees droop more than normally, presenting a somewhat wilted appearance. This appearance is exaggerated by the grayness or silvery condition of affected leaves, and the fact that in some varieties, as the Bing, the leaves are distinctly folded along the midvein.



EXPLANATION OF PLATE I

Vein clearing in Lambert cherry. 1 and 2. Leaves showing vein clearing as it appears in the orchard. 3. Symptoms of the disease produced in a leaf in the greenhouse. 4. Healthy leaf from noninoculated tree growing in greenhouse.

STEM SYMPTOMS

Cherry trees that have been affected with vein clearing for some time show a tendency to dwarf and rosette. The latter is brought about by a shortness in the internodes and an increased number of buds, spurs, or short branches at the nodes. This tendency is not evenly distributed over a tree, and, in the branches showing any rosetting, the proliferation is most pronounced at the end of the year-old wood. In a like disease affecting flowering cherries this condition is very pronounced in certain varieties.

FRUIT SYMPTOMS

Orchard trees in early stages of vein clearing may not show any noticeable malformation of fruit. In trees in which the disease is more advanced, the fruit is misshapen and reduced in size. Cherries are very pointed, flattened on the suture side, and may have a swollen ridge along the suture. Black Republican fruit shows little if any malformation. Advanced stages of the disease in the Lambert variety yield an over-abundance of blossoms, but affected trees seldom produce a normal crop of fruit. In trees of this variety in which the infection has been long-standing, fruitfulness is almost entirely wanting, even though there may be an over-abundance of flowers.

Experiments with this disease are under way, but results so far obtained in the greenhouse indicate that it is graft-transmitted and graft-perpetuated. Three trees each of Bing, Lambert, and Napoleon varieties of *Prunus avium* were grafted on February 17, 1940, and, on June 8, vein clearing was beginning to appear in leaves near the tip of two of the Lambert trees. This had become more pronounced by June 27. The appearance of symptoms was within 2 feet of the lateral branch into which the diseased scion had been inserted. In all 9 cases symptoms were perpetuated in growth from the scions.

No insect vector has been found for vein-clearing, but there are definite indications of natural inoculation and spread in orchards and nurseries.

In a comparison of the disease with mottle leaf, it may be said that, while, in vein clearing, chlorosis is essentially confined to the vascular tissues of the leaves, in mottle leaf[3] chlorosis is in the mesophyll along each side of the lateral pinnate veins but the veins remain dark. In the latter, chlorosis is much more prominent than in vein clearing. Abnormal indentation is somewhat similar in the two diseases but becomes much more severe in vein clearing. Perforation of leaves does not occur as a symptom of mottle leaf. Mottle leaf is very readily transmitted by budding or grafting. Even in cases where mere organic union of bud wood with stock took place and the buds failed to live, mottle leaf nevertheless was transmitted.

In one instance a Bing cherry tree, known to have been infected with mottle leaf for several years, showed symptoms of vein clearing on one branch in the spring of 1939. By mid-summer in 1940 mottle leaf symptoms were markedly suppressed and vein clearing was appearing in leaves on most all parts of the tree. This indicates the possible predominance of vein clearing over mottle leaf in a case of double virus infection.

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[3] Zeller, S. M. Cherry mottle leaf. Oregon State Hort. Soc. Rept. 1934: 92–95. 1934.

As to varietal susceptibility to the two diseases, the yellow varieties, Long-stemmed Waterhouse and Napoleon, are most severely affected by mottle leaf, while vein clearing affects Lambert most severely.

The common name "vein clearing" is offered for this new disease, and, in the number system, "Prunus virus 7" may be assigned to mottle leaf, which, as a common name, has come into general usage in the Pacific Northwest, and "Prunus virus 8" to vein clearing. For those using Holmes' nomenclature, vein clearing becomes *Marmor nerviclarens* and mottle leaf, *M. cerasae.*

SUMMARY

A graft-transmissible vein-clearing disease of *Prunus* has been discovered in the Pacific Northwest. It is described as a new virosis affecting cherry and prune trees. No insect vector has been found. Symptoms of vein clearing and mottle-leaf diseases of cherry are briefly compared.

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THE BORON–DEFICIENCY DISEASE OF GLOXINIA AND ITS CONTROL[1]

P. A. Ark and C. M. Tompkins

(Accepted for publication October 12, 1940)

INTRODUCTION

During the past 3 years, a serious disease of gloxinia (*Sinningia speciosa* Benth. and Hook.) has been prevalent in commercial glasshouses in the San Francisco Bay region and at Capitola, near Santa Cruz. Small to large seedlings in flats and transplanted seedlings in pots were affected, with losses frequently ranging from 50–75 per cent of the crop.

Investigations were made to determine the cause and control of this disease, a brief report of which is presented herewith.

SYMPTOMS OF THE DISEASE

Initial symptoms of the disease on young seedlings consist of small, brownish-black, non-necrotic, irregularly shaped areas at or near the base of the leaf lamina. Within a few days, the discoloration spreads toward the tip of the leaf (Fig. 1) and downward into the crown of the plant. Simultaneously, there is a marked loss in turgidity of the leaves. Frequently, the growing point of a plant is prematurely killed. Sometimes, however, seedling plants are not affected until after transplanting. On older affected plants in pots, the flowers and peduncles are considerably reduced in size

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[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

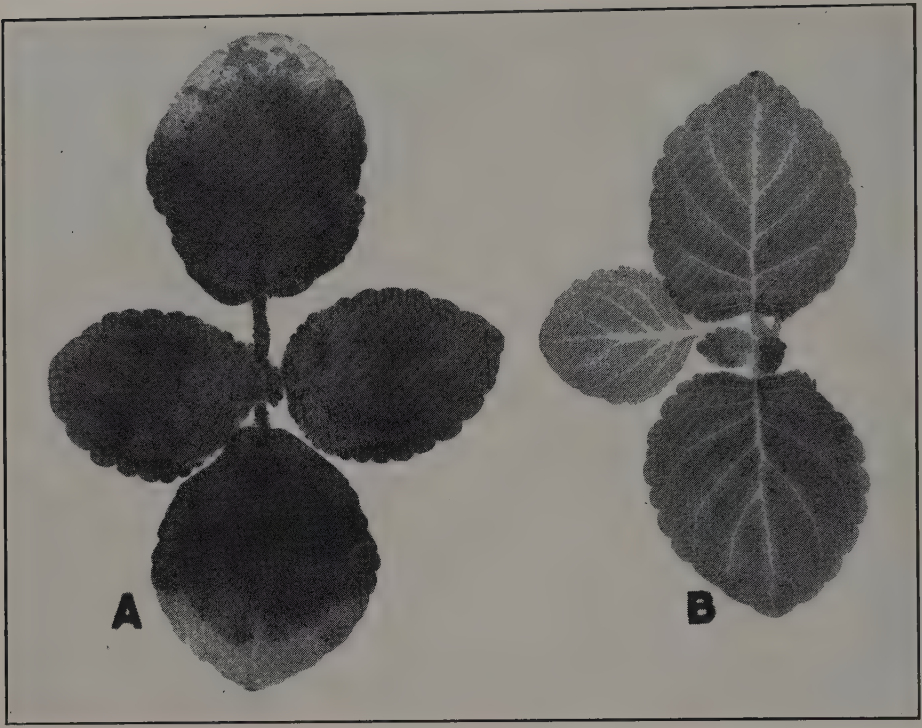

FIG. 1. Boron deficiency of Gloxinia (*Sinningia speciosa*). A. Diseased seedling, characterized by blackening of the leaf blades (from the base toward the tip) and petioles with marked loss of turgidity; B. Healthy seedling.

and also appear wilted. Eventually, the aerial parts of the plant become brown and necrotic, and the plant dies.

CAUSE OF THE DISEASE AND ITS CONTROL

Tissue plantings from leaves, petioles, peduncles, and roots of affected plants were frequently made on agar plates in the laboratory, but with negative results. The agar plates remained sterile, indicating that the causal agent of the disease was not an organism.

Attention was then given to the medium in which the gloxinia plants were grown in the nurseries. This consists of a natural compost of forest soil, twigs and debris, and rat excrement, known locally as ''ratsnest'' and obtainable in or near the coast redwood forests. Most of the ratsnest used in commercial glasshouses is taken from the forests near Occidental, Marin County, a short distance north of San Francisco. Since it has already been shown that the orchard soils at Sebastopol, near Occidental, are deficient in available boron,[2] there was reason to believe that ratsnest might be similarly affected.

Gloxinia seedling plants in various stages of the disease were taken from a nursery in San Francisco to Berkeley for experimentation. To each of 12 diseased plants contained in individual 4-in. clay pots, one application of a 6 per cent boric acid solution (making a concentration of B in the soil 0.1 p.p.m.) was applied with a watering can. Controls consisted of the same number of plants that were not treated. Within 2 weeks, newly developed leaves, petioles, peduncles, and flowers were normal in size and color, and all treated plants were well along toward complete recovery. As for the controls, the symptoms became more pronounced and, eventually, the plants died.

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[2] Ark, P. A., and H. Earl Thomas. Apple dieback in California. Phytopath. 30: 148–154. 1940.

In subsequent tests in several nurseries in San Francisco, a 6 per cent boric acid solution was applied to ratsnest in flats immediately after sowing the seed and at intervals of 2 weeks. Control flats were treated with regular tap water only. No disease developed in the treated flats in contrast to a high incidence of disease in the control flats. Similar tests were made with potted seedlings, with similar results.

The evidence indicates that ratsnest as a culture medium is deficient in sufficient available boron needed for normal growth of gloxinia plants. This deficiency can be corrected by several applications of a 6 per cent solution of boric acid applied at intervals of 2 weeks. The cost of the treatment is negligible.

A similar disease of slipperwort or pouch flower (*Calceolaria herbeohybrida* Voss) also occurs in glasshouses, when grown in ratsnest, and may be controlled by the same treatment.

SUMMARY

A serious disease of gloxinia occurs in glasshouses in the San Francisco Bay region and at Capitola.

The symptoms of the disease consist of rapid blackening and wilting of the foliage and flowers, followed by drying and death of the plant.

A similar disease has also been observed on slipperwort.

The disease is caused by boron deficiency of ratsnest, the culture medium used for these plants, and may be readily controlled by several applications of a 6 per cent solution of boric acid, applied once every two weeks.

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PHYTOPATHOLOGICAL NOTES

Incidence of Sclerotinia fructicola and S. laxa on Sweet Cherries in Oregon.[1]—In 1923 Barss[2] reported from Oregon two species of brown-rot fungi, one causing the common American brown rot and producing abundant apothecia, the other found only in its conidial or monilioid form and differing from the American brown-rot fungus strikingly in cultural characteristics. The former is now known as *Sclerotinia fructicola,* the latter as *S. laxa.* Barss stated that *S. fructicola* caused severe attacks of fruit rot on stone fruits, but might also cause blossom blight. He stated further that *S. laxa* was primarily the cause of blossom and spur blight and twig cankers, but caused little rotting of fruits.

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[1] Published with the approval of the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 368, Department of Botany.

[2] Barss, H. P. Brown-rot and related diseases of stone fruits in Oregon. Ore. Agr. Exp. Stat. Cir. 53. 1923.

In 1939 Hewitt and Leach[3] reported the results of studies on these two species of *Sclerotinia* in California. They concluded that the brown rot reported in earlier publications on this disease in California consisted almost if not entirely of the form caused by *S. laxa,* and that *S. fructicola* probably has been introduced into that State in comparatively recent years. From surveys made to determine the distribution of these two species in California they reached the conclusion that *S. laxa* has a much wider distribution than *S. fructicola* and causes most of the blossom and twig blight of stone fruits, but also causes some fruit rot. On the other hand, serious rotting of fruit has occurred in localized areas in recent years and seems to be coordinated with the introduction of *S. fructicola* into these areas.

In Oregon, in the spring and summer of 1940, in connection with some spraying and dusting experiments for the control of coccomyces leaf spot and brown rot on cherries, an incidental study of the distribution of the two species of *Sclerotinia* was made. Blighted blossoms and rotting fruits

TABLE 1.—*Distribution of Sclerotinia fructicola and S. laxa on sweet cherries in certain counties of Oregon, in 1940*

Grower	Host	Cultures from blighted blossoms		Cultures from rotten fruit	
		S. fructicola	*S. laxa*	*S. fructicola*	*S. laxa*
A. C. Berntzen Lane County	Sweet cherry	5	0	12	0
Brunk Orchard Polk County	do	3	3	0	0
Ben Dorris Lane County	do	3	0	0	0
South Farm, O.S.C. Benton County	do	0	3	0	0
A. F. Fellows Marion County	do	9	1	4	7
W. H. Gardner Marion County	do	1	5	0	11
A. F. Harvey Marion County	do	6	0	9	5
C. A. Ratcliff Marion County	do	10	7	5	5
Reynold's Estate Marion County	do	12	1	9	5
W. R. Winship Marion County	do	7	1	0	4
Totals		56	21	39	37
Percentages		72.72%	27.28%	51.32%	48.68%

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[3] Hewitt, Wm. B., and L. D. Leach. Brown-rot sclerotinias occurring in California and their distribution on stone fruits. Phytopath. **29**: 337–351. 1929.

were collected from sweet cherry trees in experimental orchards located mostly in Marion County. Included in the counts were a few cultures from material collected in two orchards in Lane County and one each in Benton and Polk Counties. The species was determined in all cases by the culture method. While the collections were very limited in number, it is believed that the results may be of enough interest to warrant this brief note.

Table 1 shows the relative percentages of *Sclerotinia fructicola* and *S. laxa* isolated from blighted blossoms and from rotted fruits, respectively. It is of special interest to note that approximately 73 per cent of the isolations from blighted blossoms yielded *S. fructicola,* while only 27 per cent gave *S. laxa.* On the other hand, the isolations from rotting fruits gave approximately 51 per cent *S. fructicola* and 49 per cent *S. laxa,* or very nearly equal percentages of the two species. These figures, while based upon a very limited count, are, nevertheless, at variance with statements made both by Barss and by Hewitt and Leach.

It is intended to extend these studies to peaches, plums, and apricots as the opportunity arises. At the present time material is being collected from peaches.—A. W. EVANS and C. E. OWENS, Oregon Agricultural Experiment Station, Corvallis, Oregon.

Single-spored and Monosporous vs. ....?—In order to express the fact that a culture of an organism was derived from a single reproductive entity (spore, conidium, cell) authors have employed several different words, as monosporous, monosporic, monosporial, and single-spore(d). Are they strictly synonymous?

Probably *monosporous* has been most widely used with the intended significance of derived from, but not composed of or bearing, one spore. The dictionary definition (Webster's New International), however, restricts it to the latter sense, *i.e.,* monosporiferous. The suffix *-ous* means literally *full of* or *possessing.* A suffix that more precisely expresses origin or derivation is *-ic* which means *of or pertaining to*; the suffix *-al* (*-ial*) means almost the same with the additional connotation *of belonging to.* Either *monosporic* or *monosporial* would precisely denote something belonging or pertaining to one spore, but neither word as yet has dictionary sanction. In one respect *monosporic* is preferable in that the prefix and suffix are both of Greek origin as is also the root, whereas the suffix *-al* is Latin. *Monosporic* would also agree in form with *monocytic,* which might be useful in certain connections. On the other hand, *monosporial* would parallel *monoconidial,* thus facilitating observance of the mycological convention that restricts *spore* to a reproductive structure of the perfect or sexual stage of an organism and *conidium* to the imperfect or asexual stage. "Monoconidic" appears impossible.

Possibly those who prefer "English" words will continue to use the nounal adjective *single-spore,* or the participial adjective *single-spored,* but these expressions can mean literally either derived from or containing one spore. Besides, it is to be feared that printers and editors in their crusade against hyphens would eliminate this one and write the word *singlespored,* which only looks strange, whereas its logical counterpart *singleconidial* is definitely forbidding.—FREEMAN WEISS, Bureau of Plant Industry, Washington, D. C.

ANNOUNCEMENT

SUMMER MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The summer meeting of The American Phytopathological Society will be held jointly with the newly organized New England Division of the Society from June 24 to June 27, inclusive. Headquarters will be the Connecticut Agricultural Experiment Station in New Haven. The program aims to show visitors the phytopathological problems and facilities of a cross section of New England. The tentative program is as follows:

June 24—10:00 A.M. assemble at Bartlett Tree Research Laboratories, Stamford, Conn. *Subject:* Tree Diseases. 12:30 P.M. Clambake, courtesy of the F. A. Bartlett Tree Expert Company. 2:00 P.M. Leave for New Haven and tour of Yale University. 6:00 P.M. "Soup's-On" by Chef Stoddard. 7:30 P.M. Open house at the Connecticut Agricultural Experiment Station. Party for the ladies.

June 25—8:30 A.M. Laboratories of Forest Pathology, U.S.D.A., Marsh Hall, Yale University. 10:30 A.M. Experiment Station Farm, Mt. Carmel (Conn.), fruit and vegetable diseases, organic fungicides. 12:30 P.M. Picnic lunch at Sleeping Giant State Park. 4:00 P.M. Arrive at Tobacco Sub-station, Windsor. 7:00 P.M. Panel discussion or technical papers. 9:00 P.M. Smoker.

June 26—8:30 A.M. Visit to shade-tobacco farm and curing sheds. 1:00 P.M. inspection of greenhouse and field experiments at Waltham (Mass.) Field Station. 5:30 P.M. arrive at Durham, New Hampshire, to join in A.A.A.S. program. Complimentary concert tickets as guests of University of New Hampshire.

June 27—Join with Horticultural and Plant Physiological societies for papers of mutual interest. The nearby White Mountains and the Maine Coast may serve as a vacation objective.

The meeting is planned to provide everyone an opportunity to give and to receive information, ideas, and pleasure. Informality is the keynote. The arrangements committee would appreciate word of your interest in attending, suggestions for the good of the program, and titles of papers (10 minutes' limit). Abstracts of papers to be presented must conform to the editorial policies of Phytopathology and be mailed to the Division secretary by June 2. Come prepared to see and enjoy New England and to help launch a new Division of our ever-advancing Society.

Frank L. Howard, Secretary,
New England Division,
Kingston, Rhode Island.

Arrangements Committee:

R. J. Anderson
J. S. Boyce
E. F. Guba
R. P. Marshall
M. C. Richards
E. M. Stoddard
J. G. Horsfall, Chairman.

Photograph by Bachrach

JAMES FRANKLIN COLLINS
(1863–1940)

JAMES FRANKLIN COLLINS

WALTER H. SNELL AND ALMA M. WATERMAN

Professor J. Franklin Collins, taxonomist and forest pathologist, died in Cranston, R. I., on November 14, 1940, after a long illness.

Professor Collins was born December 29, 1863, in North Anson, Maine. He moved to Providence in 1873, where he was educated in the grade and high schools. From 1879 to 1898 he was a silver worker, designer, and embosser for the nationally known Gorham Manufacturing Company. He early became interested in collecting and identifying plants, and soon came into contact with Professor W. W. Bailey, Head of the Department of Botany at Brown University. Young Collins immediately began to collect and study plants assiduously under Professor Bailey's guidance. In 1894, while still working at his trade at Gorham's, he was appointed Curator of the Olney Herbarium at Brown University, and in 1896 was awarded an Honorary Ph.B. degree by that institution. In 1899, he gave up his work as a silversmith and undertook the duties of Instructor in Botany at Brown. He was made an Assistant Professor in 1905 and upon Professor Bailey's retirement in 1906, he became Head of the Department, in which position he continued until 1911.

In 1907, Collins started on another tack, which was to develop into the main work of his later life. From that date, until 1911, while still teaching at Brown, he became successively Collaborator, Agent, Special Agent, and Forest Pathologist in the Office (Division) of Forest Pathology of the U. S. Department of Agriculture, working chiefly upon the chestnut-bark disease. Then, in 1913, when the late Dr. Haven Metcalf established a branch laboratory of the Office of Forest Pathology at Brown University to deal with the diseases of shade and ornamental trees and shrubs, Collins was placed in charge. He was subsequently appointed Pathologist and Senior Pathologist-in-Charge. He did not give up his interest in taxonomy at any time during this period, but served as Curator of the Olney Herbarium and as Demonstrator and Lecturer in Botany until he retired from the government service in 1933.

Collins' own botanical interests were broad. He was an expert in the higher plants, ferns, and mosses. He had a moderate knowledge of the fungi. He taught courses in taxonomy, morphology, histology, physiology, and ecology of the seed plants and higher cryptogams, medical botany, mycology, and some plant pathology. After his early investigations, connected with chestnut blight, he turned his energies to problems of the care and treatment of wounds in shade trees. His solid accomplishments in this field, at that time pervaded with fake and quackery, earned him the title of "Father of Tree Surgery." He was responsible for four improvements in the protection of shade trees: 1. Scientific methods of trimming, etc., instead of the crude tree butchery too commonly practiced. 2. The open cavity, properly made. 3. A wood-filler for cavities instead of the inelastic cement. 4. A sawdust-asphalt mixture for a more flexible filling. He had the two latter cavity-fillers patented by the U. S. Department of Agriculture to make the methods available to the public. His Farmers' Bulletin 1178 on "Tree Surgery" ran through 9 editions and revisions, from 1920 to 1934, with a total of 210,000 copies, and Farmers' Bulletin 1726, "Treatment and Care of Tree Wounds," 5 editions, from 1934 to the present time totalling 160,000 copies.

Collins had considerable acquaintance with plants over the country. He had a broad and intimate knowledge of the plants of Rhode Island. He knew every corner of the State, every crossroad and brook. He collected widely with other members of the New England Botanical Club, more especially in Maine and the Gaspé Peninsula. In the Gaspé region he did a great deal of exploring of hitherto untrod territory and because of his exploration of one mountain, that peak now bears the name of "Mount Collins" and is so accepted by the Canadian Geological Survey. In his later work for the Government, he traveled considerably in this country to study trees and their diseases, parks, and forests.

Collins' publications numbered over 100, with about a score each on mosses and chestnut blight, and the others on ferns, local floras and miscellaneous higher plants. Outside of his two bulletins on the care and treatment of shade trees, his best-known work was "Key to New England Trees, Wild and Cultivated," with Howard W. Preston.

Collins was a member of the following professional organizations: American Association for the Advancement of Science (Fellow), American Forestry Association, The American Phytopathological Society (charter member), Botanical Society of America, Josselyn Botanical Society of Maine (Chairman of Bryophyte Committee for 10 years), National Geographic Society, New England Botanical Club (Committee on Check List 10 years), Rhode Island Botanical Club (one of founders and president several years), Rhode Island Field Naturalists Club (president 3 years, and member of executive Committee 4 years), Rhode Island Horticultural Society, Sigma Xi, (Brown University Chapter—Treasurer 2 years), Sullivant Moss Society, Torrey Botanical Club.

Collins was a typical Yankee genius in manual accomplishments. Trained as a worker in the precious metals, he could turn carpenter, machinist, plumber, tinsmith, or electrcian with equal facility. With some sheet tin or brass, a little wood, some solder, a few nails or screws and the most ordinary of tools, he could make unbelievable things. His small office was only a cubicle, but he made it serve at once as office, personal laboratory, library, herbarium, and workshop. The small laboratory that he directed provided twice the facilities the space would seem to justify, because of his ability to devise gadgets and arrangements of all sorts that saved valuable space and that packed in two sets of equipment where there was room for only one. He actually made the old physical saw that two objects cannot occupy the same space at the same time, appear ridiculous. Pull down a few black curtains from their hiding place near the ceiling, release a few catches and locks, lower or unfold some collapsible shelving, put a few devices of his own invention in the nearby sink, and one had a photographic dark-room with all necessary equipment in a corner where a dozen other operations had been carried on only a short time before. He was an accomplished photographer, a skillful bookbinder, and an artist at preparing and mounting herbarium specimens. He was not unhandy with pen and pencil.

Professor Collins was quiet, modest, reserved, but very kindly and companionable when one got to know him. He knew when to listen and when to speak. He possessed a twinkling humor. He had a remarkable memory for names, places, and facts. His sagacious and practical advice was always comforting, as well as enlightening. He was abstemious and rather spartan in his own life, but had a personal warmth that was often unsuspected. He possessed that attribute called "horse sense" that is so often found in a "State-of-Mainer." He viewed life philosophically, and his last illness, which he saw beginning to fasten its hold upon him ten years ago, he viewed with equanimity, profound courage and detachment, as he observed its course with a clear mind to the very end.

THREE SPECIES OF PYTHIUM WITH PROLIFEROUS SPORANGIA

CHARLES DRECHSLER

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INTRODUCTION

Some years ago I (6) published diagnoses of 15 new species of *Pythium* that had been isolated from diseased roots and other decaying plant structures. More recently (9, 10, 11) the diagnoses of 9 of these species have been supplemented with figures and discussion setting forth various details of morphology in a somewhat comparative manner. In the present paper supplementary comment will be given to 2 of the remaining species, namely *P. oedochilum* and *P. palingenes,* both of which were made known as proliferous forms closely related to *P. helicoides* Drechsl. To bring the distinctiveness of these forms into appropriate relief, occasion is taken to describe as new a congeneric species, which, though showing sporangial proliferation, is manifestly alien to the *helicoides* series. The figures illustrating sexual reproduction in the 3 fungi are given at a magnification (*i.e.,* ×1000) uniform with that of similar figures in previous papers. Owing to spatial necessities the zoosporangia and zoospores are shown in drawings reproduced at the lesser magnification (*i.e.,* ×500), previously employed in illustrations of similarly rangy asexual apparatus.

PYTHIUM OEDOCHILUM

The description of *Pythium oedochilum* was based on 4 cultures isolated from separate portions of decaying tissue excised from the stem and larger roots of a dahlia (*Dahlia rosea* Cav.) plant found seriously affected with crown rot in the District of Columbia, late in August 1926. Two weeks of continuous wet weather had led to unusual prevalence of root rot in many cultivated and many wild herbaceous plants, much of the decay being due apparently to oomycetous parasites not frequently encountered under more normal conditions. The fungus came to light subsequently from another source, on being isolated in pure culture from discolored roots of 3 specimens of *Bidens aristosa* (Michx.) Britton, collected near Broad Run, Virginia, in September 1931.

Growing on maize-meal agar in plate cultures, *Pythium oedochilum* shows a rate of mycelial extension about median between that of the slower-growing members of the genus, as, for example, *P. complens* Fischer, and that of the faster-growing members such as *P. ultimum* Trow and *P. butleri* Subr. Its hyphal branches, on coming in contact with the wall of a glass container, expand terminally to form bulbous or clavate appressoria (Fig. 1, A), which in most instances elongate into curved or slightly branched structures (Fig. 1, B, C). As the result, presumably, of functional frustration inevitable under the circumstances, the curved appressoria may further give rise to irregularly sinuous hyphal outgrowths (Fig. 1, D, E).

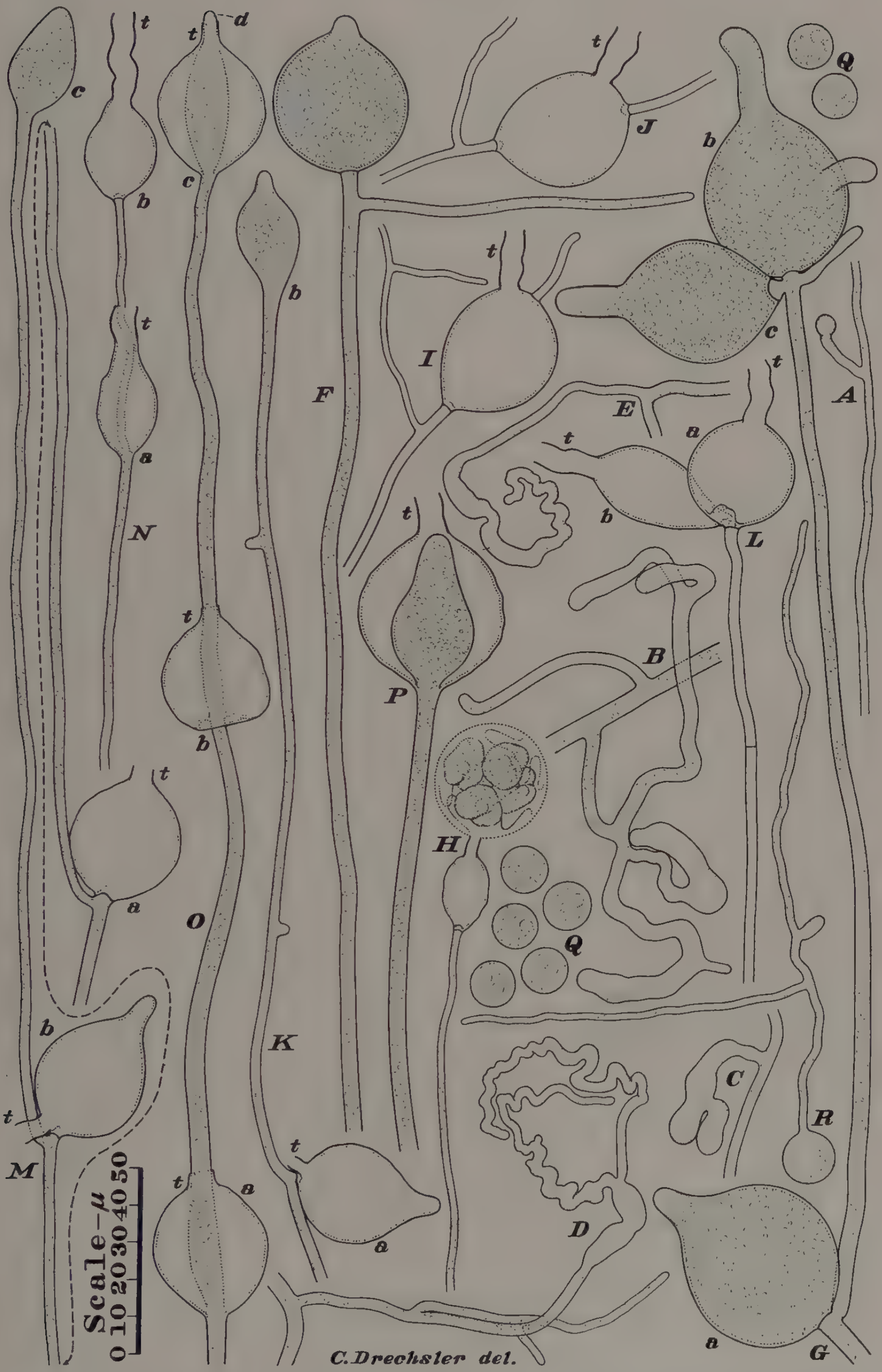
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FIG. 1. Strain of *Pythium oedochilum* isolated from dahlia root, drawn with the aid of a camera lucida; ×500 throughout. A–E. Appressoria. F–Q. Asexual reproductive stage; t, evacuation tube. From lack of space M is shown in parts connected by a broken line.

Only rather scanty aerial mycelium is ordinarily formed on maize-meal agar in Petri dishes, though on the same medium in tubes the fungus produces a substantial cottony mat, which, like the cottony mat of *P. helicoides,* often persists for years without collapsing to the substratum.

Asexual reproduction may conveniently be induced in *Pythium oedochilum* by excising from a thinly poured Lima-bean-agar plate culture small slabs of medium well permeated with young mycelium, and transferring them to a shallow layer of water in a sterile Petri dish. Each tract of substratum thus placed under aquatic conditions soon extends into the surrounding water a fringe of filaments several millimeters in length. These submerged filaments show in their distal portions little of the racemose or corymbose branching characteristic of the submerse hyphae formed in irrigated preparations of *P. helicoides.* However, the sporangia which they produce terminally resemble those of *P. helicoides* rather closely, since, for the most part, they are subspherical, ovoid, or obovoid, and bear a papillate protrusion at the apex. In a stale preparation such a papillate sporangium may undergo no marked change for weeks, except that a central vacuole appears after a few days, and gradually increases in size with the passage of time. If staling is prevented by changing the water at intervals, the sporangial contents are discharged into a vesicle (Fig. 2, A, a), and in 20 to 25 minutes are transformed into biciliate zoospores wholly after the manner usual in the genus *Pythium* (Fig. 1, H; Fig. 2, A, b). In the meantime, the supporting hypha frequently resumes development to form a second sporangium within the empty envelope of the first (Fig. 2, A, a–d). Apart from instances of proliferation that result in a nested arrangement (Fig. 1, O, d; P; Fig. 2, E, b), uniaxial replication is accomplished also when the supporting hypha continues growth through the empty envelope as a filament and produces a subsequent sporangium externally, whether in a position sessile on the empty evacuation tube (Fig. 2, B, b) or in a more forward position (Fig. 1, N, b; O, b, c; Fig. 2, D, b, c). Sometimes, especially when dehiscence of a sporangium is delayed so that uniaxial prolongation of the supporting hypha cannot take place, this hypha grows out laterally below the sporangial septum and gives rise to a subsequent sporangium, either close by (Fig. 1, G, c; L, b) or farther on (Fig. 1, G, b; K, b; M, b, c; Fig. 2, C, b; D, b; E, c). Filaments bearing 3 or more sporangia may reveal only uniaxial prolongation (Fig. 1, O), or only subsporangial branching (Fig. 1, G; M), or they may show both types of proliferation (Fig. 2, D; E). Replicative development of either kind is found much less frequently associated with the sporangia that come to be produced, often rather abundantly, in intercalary (Fig. 1, J) or subterminal (Fig. 1, I; Fig. 2, F, a, b; G, b) as well as in terminal (Fig. 2, G, a) positions on the tracts of mycelium present in the agar medium before its transfer to water.

The relatively small apical protrusion, which, as has been stated, is usually put forth by the sporangium when it reaches definitive size (Fig. 1, F; Fig. 2, C, b; E, c), sometimes serves in dehiscence without much outward

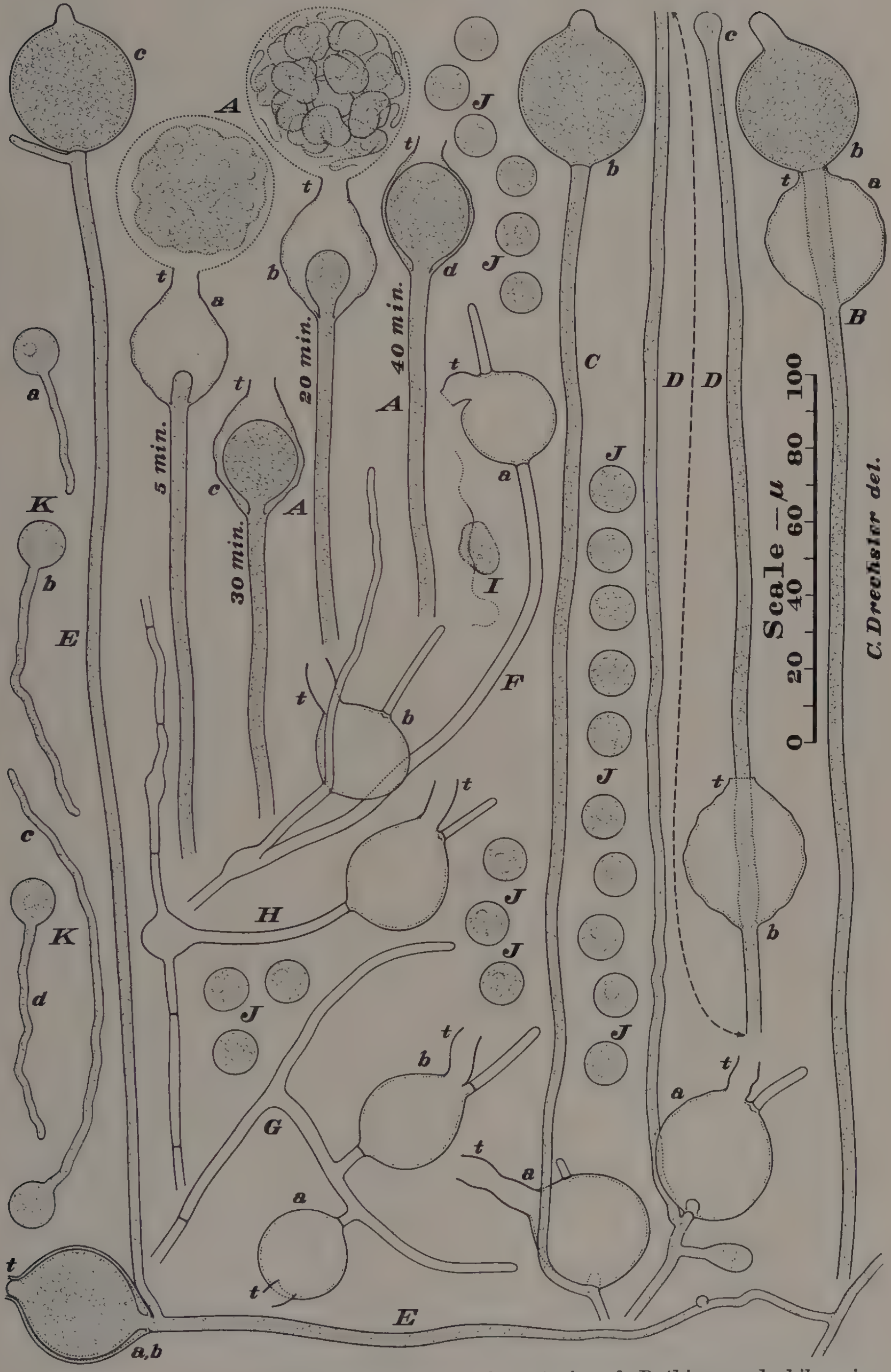
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FIG. 2. Asexual reproductive apparatus of a strain of *Pythium oedochilum* isolated from *Bidens aristosa*, drawn with the aid of a camera lucida; ×500 throughout; t, evacuation tube. From want of space D is shown in parts connected by a broken line.

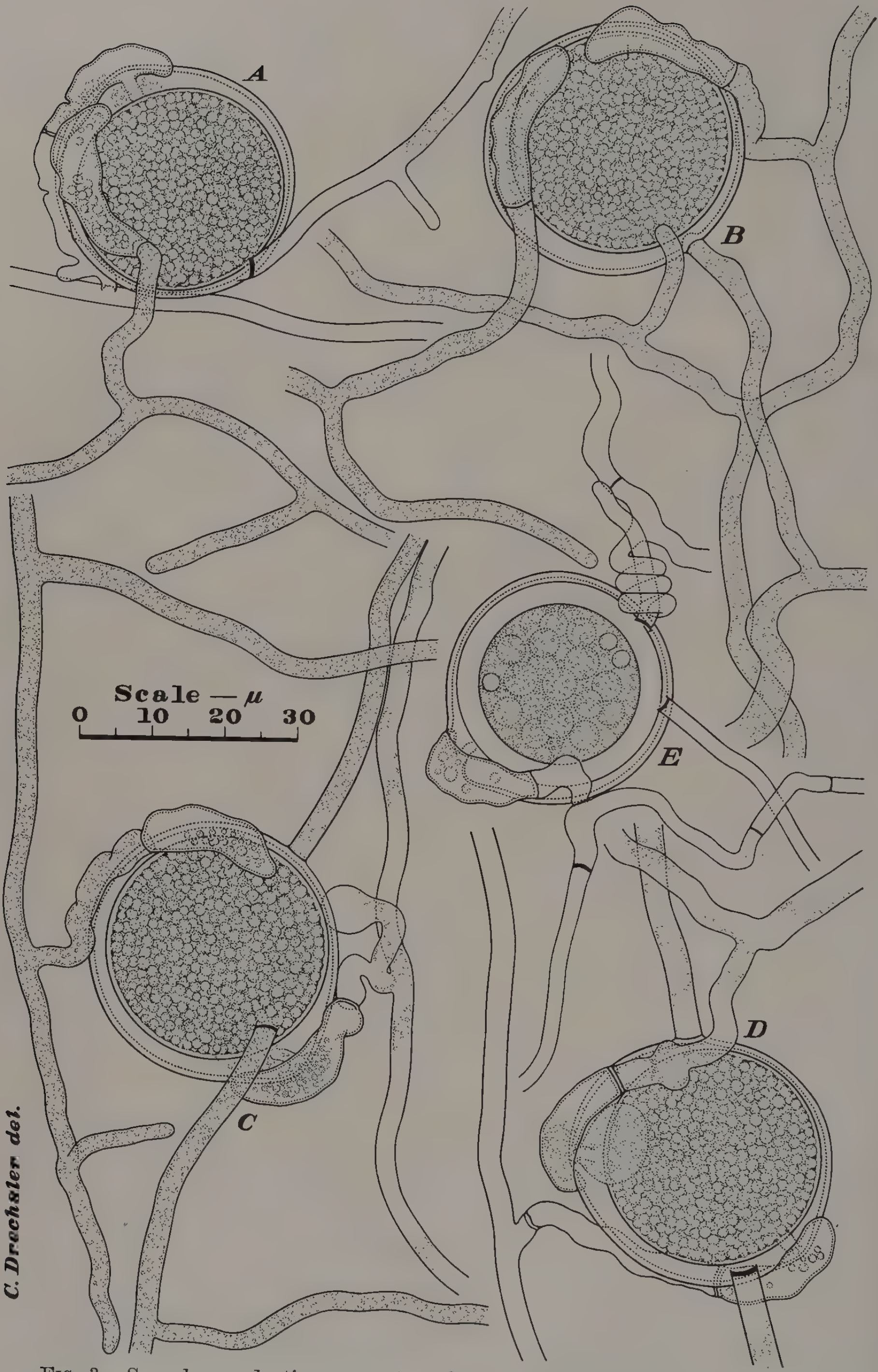
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FIG. 3. Sexual reproductive apparatus of a strain of *Pythium oedochilum* isolated from dahlia root, drawn with aid of a camera lucida; ×1000 throughout.

change; the empty sporangial envelope in such instances showing only a rudimentary collar (Fig. 2, B, a; D, b). More often, however, the protrusion grows out into a cylindrical process (Fig. 1, G, a, c; Fig. 2, B, b) before it yields at its tip to permit escape of the protoplasmic contents; the emptied sporangial envelope in these more numerous instances bearing an evacuation tube of appreciable length (Fig. 1, H; L, a, b; N, a, b; O, a, b, c; P). When an apical papilla or apical process suffers functional frustration, a tube put forth from some other position (Fig. 1, G, b) operates in the discharge of the sporangium (Fig. 1, K, a; M, b). Intercalary and subterminal sporangia more often discharge through an evacuation tube arising from a forward position than through one arising from an equatorial or proximal position (Fig. 1, I; J; Fig. 2, C, a; D, a; F, a, b; G, a, b; H).

After being liberated by disintegration of the vesicle wherein they are formed, the laterally biciliate zoospores (Fig. 2, I) swim about for some time, and then round up into subspherical cysts (Fig. 1, Q; Fig. 2, J). These cysts germinate by producing usually a single germ tube (Fig. 1, R; Fig. 2, K, a–d).

Sexual reproduction of *Pythium ocdochilum* takes place abundantly in maize-meal agar cultures and with very little degeneration. The oogonia, spherical in shape, often are formed terminally on hyphae of variable lengths (Fig. 3, A; B; Fig. 4, A–C; E–H; I; Fig. 5, A; B; D; F), though, often again, they are found in intercalary (Fig. 3, D; C; Fig. 5, C) or subterminal (Fig. 3, E; Fig. 4, D) positions. They are supplied mostly with 1, 2, or 3 elongated, somewhat expanded antheridia that are borne terminally on rather sturdy hyphal branches. Ordinarily, the mycelial connection between male and female organs, while necessarily present in cultures containing only a single thallus, is too remote to be traceable; yet monoclinous sexual units occur here and there (Fig. 4, E; Fig. 5, E; F), especially in irrigated preparations, where some extramatrical hyphal filaments may be so widely separated from their fellows that interaction is appreciably reduced. Spiral involvement of the oogonial hypha by an antheridial branch belonging to the same sexual unit—a most consistent feature of *P. helicoides*—occurs also in this species (8, Fig. 4, G), but only very rarely. Now and then an oogonial filament may be found enwrapped spirally by a hyphal branch not closely connected with any male organ (Fig. 3, E; Fig. 5, A); and, occasionally, helicoid involvement is observable where neither of the elements concerned has any close connection with a sexual structure (Fig. 5, G).

In *Pythium oedochilum,* as in *P. helicoides,* the elongated antheridium is applied lengthwise to the oogonium. Often, especially when only one male organ is present, the oogonium protrudes rather markedly along the line of contact, the protrusion being indented longitudinally to provide a groove into which the antheridium is partly countersunk. The fertilization tube comes from a median or slightly forward position on the antheridium, where this organ is set most deeply in the groove; so that the indented protuberance and the tube together frequently present a thick-lipped profile

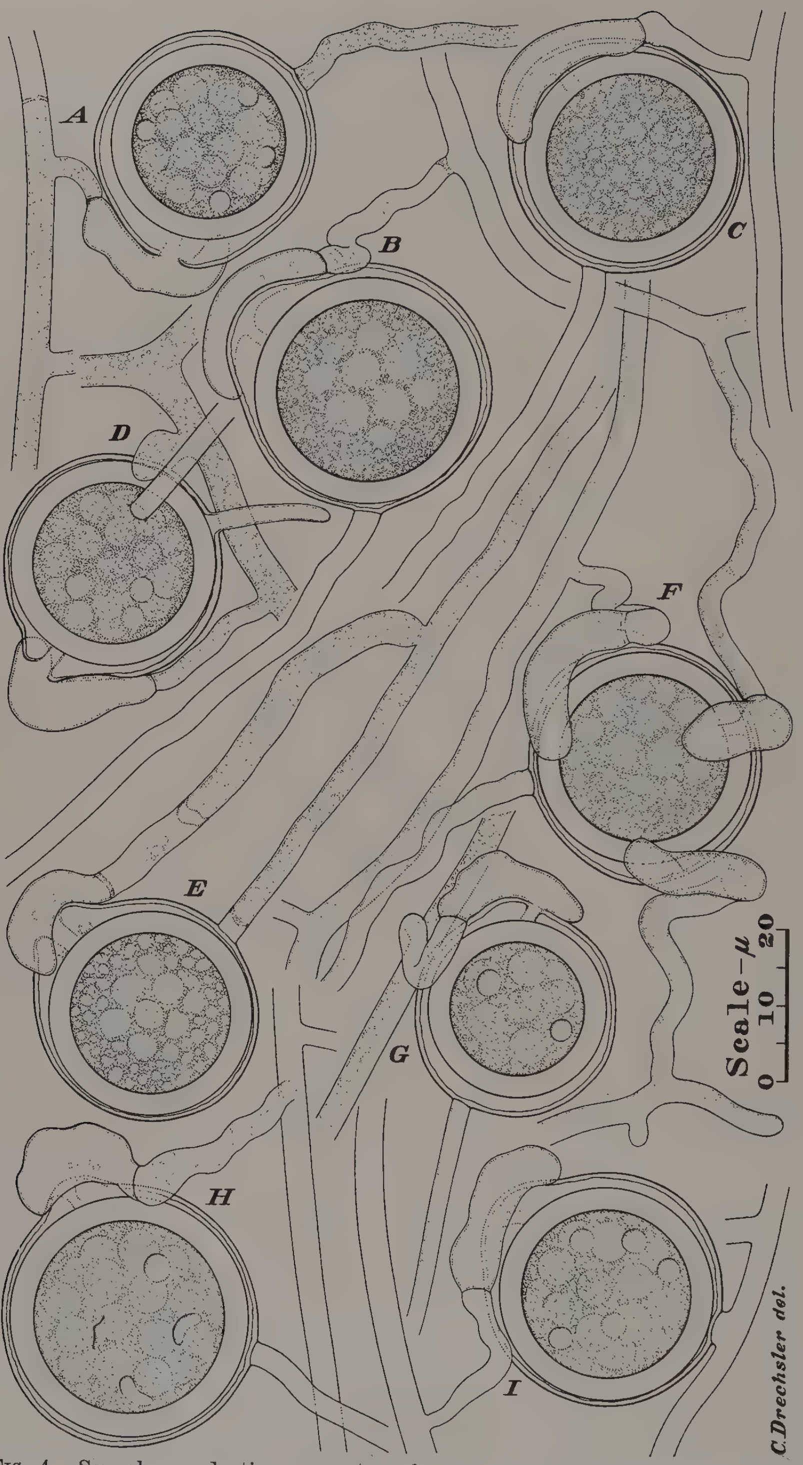
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FIG. 4. Sexual reproductive apparatus of a strain of *Pythium oedochilum* isolated from dahlia root, drawn with aid of a camera lucida; ×1000 throughout.

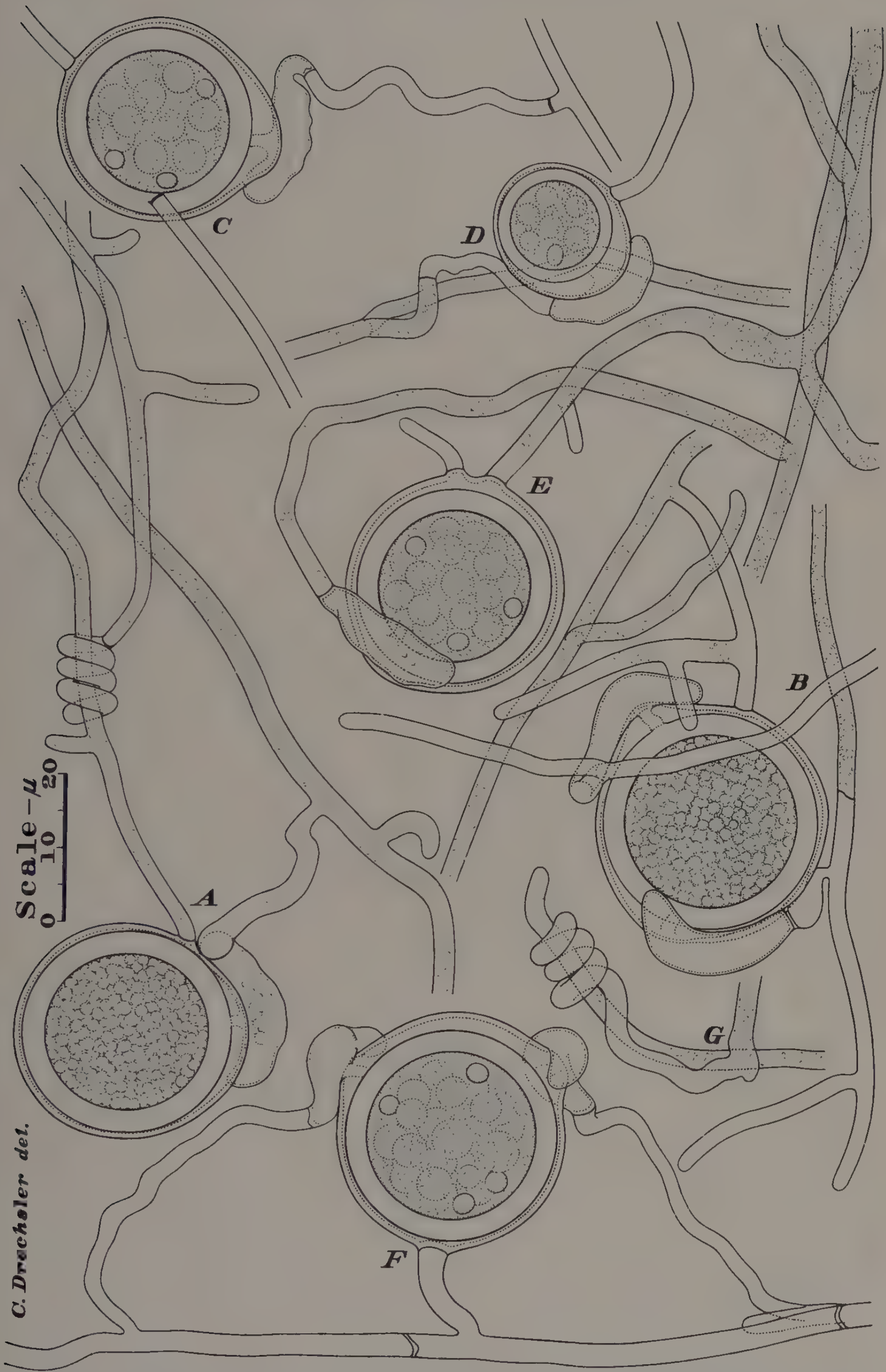
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FIG. 5. Sexual reproductive apparatus of a strain of *Pythium oedochilum* isolated from *Bidens aristosa,* drawn with aid of a camera lucida; × 1000 throughout.

(Fig. 3, E; Fig. 4, D; E; H), which indeed suggested the specific name given to the fungus, a term compounded from two words meaning "to swell," and "lip," respectively. When fertilization has taken place the oogonial contents contract into a spherical body filled throughout with numerous globules of rather uniformly small size (Fig. 3, A–D). Soon this body becomes surrounded by a thick wall of its own (Fig. 5, A, B). As its globulose contents undergo further gradual conversion (Fig. 4, B; C; E; F) an oospore is brought into being that at early maturity contains 5 to 20 reserve globules and 3 to 4 smaller refringent bodies (Fig. 3, E; Fig. 4, A; D; G; H; I; Fig. 5, C–F). With additional maturation the refringent bodies often show some increase in number. The multiplicate internal structure of its oospores clearly marks the fungus as being closely related to *P. helicoides.*

The sexual apparatus of *Pythium oedochilum,* produced abundantly in maize-meal agar, is not prone to excessive variation with respect to its main dimensions. Thus the 200 measurements for diameter of oogonium, from which was computed the average of 31.5 μ given for this dimension in the diagnosis of the species, showed a distribution of values (expressed to the nearest integral number of microns) as follows: 19 μ, 1; 24 μ, 2; 25 μ, 2; 26 μ, 3; 27 μ, 9; 28 μ, 7; 29 μ, 7; 30 μ, 24; 31 μ, 34; 32 μ, 31; 33 μ, 26; 34 μ, 29; 35 μ, 13; 36 μ, 8; 37 μ, 3; 39 μ, 1. And the 200 measurements for diameter of oospore, from which was computed the average of 28.1 μ, likewise given in the diagnosis, showed the following distribution of values: 16 μ, 1; 21 μ, 1; 22 μ, 2; 23 μ, 5; 24 μ, 9; 25 μ, 9; 26 μ, 12; 27 μ, 29; 28 μ, 35; 29 μ, 40; 30 μ, 24; 31 μ, 17; 32 μ, 9; 33 μ, 5; 34 μ, 2. As the strain from dahlia roots was the only one known when the species was described, the metric data then given were derived from cultures of that strain. Closely comparable distributions are shown in the sets of values obtained by measuring the oogonia and oospores in 200 units of sexual apparatus selected at random in a maize-meal agar culture of the strain isolated from *Bidens aristosa.* Here the values for diameter of oogonium, which gave an average of 31.9 μ, were distributed as follows: 24 μ, 1; 26 μ, 1; 27 μ, 1; 28 μ, 2; 29 μ, 5; 30 μ, 28; 31 μ, 42; 32 μ, 46; 33 μ, 34; 34 μ, 25; 35 μ, 9; 36 μ, 5; 37 μ, 1; while the values for diameter of oospore, from which an average of 28.1 μ was computed, had the following distribution: 19 μ, 1; 24 μ, 4; 25 μ, 3; 26 μ, 14; 27 μ, 45; 28 μ, 53; 29 μ, 41; 30 μ, 26; 31 μ, 11; 32 μ 1; 33 μ, 1. The 200 values for thickness of oospore wall obtained in the measurements made on the strain from *B. aristosa* gave an average of 2.9 μ, as compared with the average of 2.5 μ earlier computed from corresponding measurements of the dahlia strain. Apparently this rather marked dimensional difference is traceable to differences in cultural conditions rather than to morphological dissimilarity between the two strains.

PYTHIUM PALINGENES

The description of *Pythium palingenes* was based on 2 cultures isolated from darkly discolored roots of a giant ragweed (*Ambrosia trifida* L.) plant that was taken up from a moist roadside near Delaplane, Virginia, late in August 1926. Cultures of the fungus were obtained also from discolored roots of giant ragweed plants collected 3 weeks later near a watercourse in the District of Columbia. In both localities development of root-rotting parasites had been encouraged by a period of wet weather preceding the collection of the infected material.

Growing free of contaminating organisms, at a temperature of 24° C., *Pythium palingenes* extends its mycelium more rapidly than *P. oedochilum,* though somewhat less rapidly than *P. helicoides.* Its submerged mycelium in maize-meal agar cultures presents to the naked eye a diffuse appearance without noticeable luster; and, accordingly, reveals under the microscope promiscuous rather than parallel arrangement of hyphal parts. Since the vegetative filaments vary in width between 2 and 7 μ, the fungus is of approximately the same texture as the widely familiar *P. debaryanum* Hesse. On encountering the glass wall of a Petri dish the more delicate branches often give rise to terminal appressoria, clavate or knob-like in shape. Tracts of aerial mycelium often partly clothe the surface of the substratum in plate cultures, while in tube cultures, as a rule, the medium is wholly covered with a weft of cottony growth, which, under ordinarily favorable conditions for storage, persists for years without collapsing.

Material suitable for inducing abundant asexual reproduction in *Pythium palingenes* is readily obtained by growing the fungus on Lima-bean agar in thinly poured plate cultures. When slabs, well permeated with vigorously growing mycelium, are excised from such cultures and transferred aseptically to a sterile Petri dish containing a layer of water deep enough just to cover the medium sparingly, filaments often more than 1 or 2 mm. in length grow out into the water to produce terminally a subspherical or ovoid sporangium provided with an apical papilla. As the long filaments are largely devoid of branches in their distal portions, the primary sporangia here, like those of *P. oedochilum*, lack the corymbose or racemose arrangement frequent in *P. helicoides.* Accordingly, proliferous development here, much as in *P. oedochilum,* eventuates mainly in unilinear series. A sporangiferous filament, after growing through the empty envelope of one sporangium, produces another, either some distance onward (Fig. 6, A, a, b, c, d; B, a, b, f), or directly superimposed on the empty evacuation tube (Fig. 6, C, a, b). Very often, too, a new sporangium is formed within the empty envelope of an old one, so that a nested arrangement is brought about (Fig. 6, A, e; B, c, e, g; C, c; E, a, b, c; G, a, b). Sometimes, again, where dehiscence of a sporangium is delayed, the supporting hypha grows out below the delimiting septum to produce another sporangium, whether at a distance (Fig. 6, B, d) or close by (Fig. 6, D, a, b).

The dome-shape apical papilla, usually present on a sporangium when it attains definitive size, may function directly in dehiscence (Fig. 6, A, b; B, f; C, b; E, a), though often it grows out into an evacuation tube of some length before the sporangial contents are expelled into a vesicle formed at

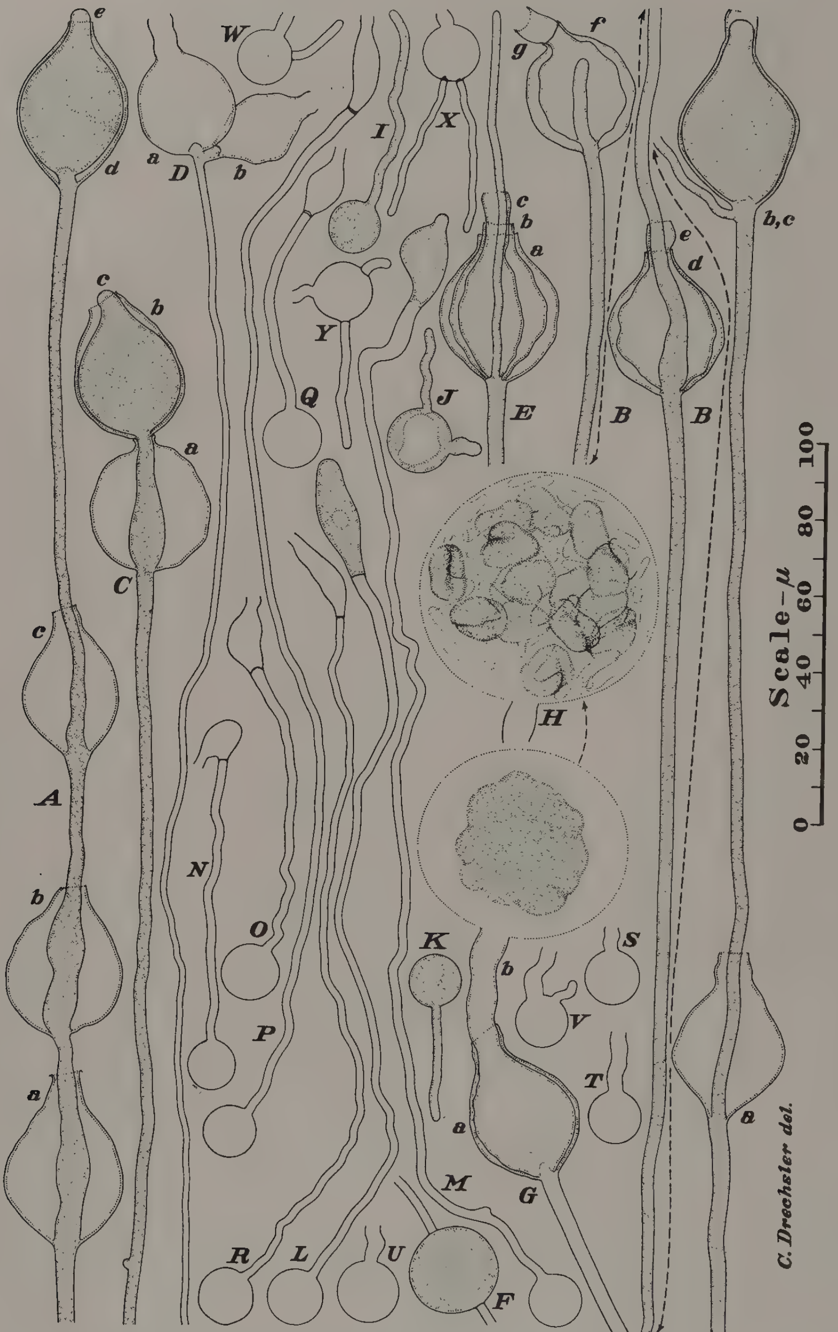
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FIG. 6. Asexual reproductive stage of *Pythium palingenes*, drawn to a uniform magnification from an irrigated Lima-bean-agar preparation with the aid of a camera lucida; ×500 throughout. From want of space B is shown in 3 parts connected by broken lines. H represents a more advanced stage of the vesicle shown in G.

its tip (Fig. 6, G, b). Within this vesicle the undifferentiated mass of protoplasm is fashioned into laterally biciliate zoospores wholly after the manner typical of *Pythium* (Fig. 6, H). On escaping from the vesicle the zoospores, among the largest known in the genus, swim about for a time before they come to rest and round up into cysts having an average diameter of 14 µ. The cysts may germinate vegetatively by 1 or 2 germ tubes (Fig. 6, I, J, K). However, in irrigated preparations, which through repeated renewal of water have been kept fresh as well as moderately free of dissolved nutrients, germination of zoospores often takes place by production of longish sporangiferous filaments whereon are borne miniature sporangia (Fig. 6, L, M) that give rise to single secondary zoospores, morphologically similar to the primary ones, though of smaller size. Besides the numerous empty units consisting individually of the membranous remains of zoospore, sporangiophore, and sporangium (Fig. 6, N–R), irrigated preparations often show numerous empty zoospore envelopes, each provided with a broad empty evacuation tube open at its tip (Fig. 6, S, T, U). Frequently these envelopes, which obviously testify to the development of an iterated swarming stage by emission of protoplasmic contents into a vesicle directly from the encysted zoospore, bear, in addition, 1 or 2 narrow empty processes, in some instances continuous with the cyst (Fig. 6, V), in other instances set off by a cross-wall (Fig. 6, W, X, Y). Presumably these processes represent membranous remains of short germ sporangiophores that became abortive when superseded by the successfully functional evacuation tubes. From the repeated emergence and renewed animation so freely displayed by its zoospores, the fungus was given a specific name defined in the familiar phrase "born again."

Subspherical bodies apparently homologous with zoosporangia are produced in rather large numbers on aerial mycelium overlying agar cultures of *Pythium palingenes*. For the most part they are borne terminally on delicate filaments or branches, though intercalary specimens are not rare (Fig. 6, F). When borne terminally they are usually provided with a small apical papilla. Despite this modification for emission of contents, the bodies on being placed in water develop much more frequently by putting forth germ tubes than by discharging their protoplasmic materials into a vesicle for transformation into zoospores.

Sexual reproduction takes place abundantly and with very little degeneration when *Pythium palingenes* is grown in pure culture on maize-meal agar containing a small quantity of finely divided maizemeal. The subspherical oogonia are often borne terminally, either on relatively long hyphae (Fig. 7, A; Fig. 8, A, C, E) or on shorter branches (Fig. 7, B; Fig. 8, D). Often, too, they arise as intercalary bodies; in many instances of such origin coming to have, when fully grown, a tangential or laterally sessile attachment (Fig. 7, C; Fig. 8, B). Lateral, as distinguished from mesial, attachment is similarly frequent with subterminal oogonia (Fig. 7, D). The antheridia are for the most part borne terminally on branches

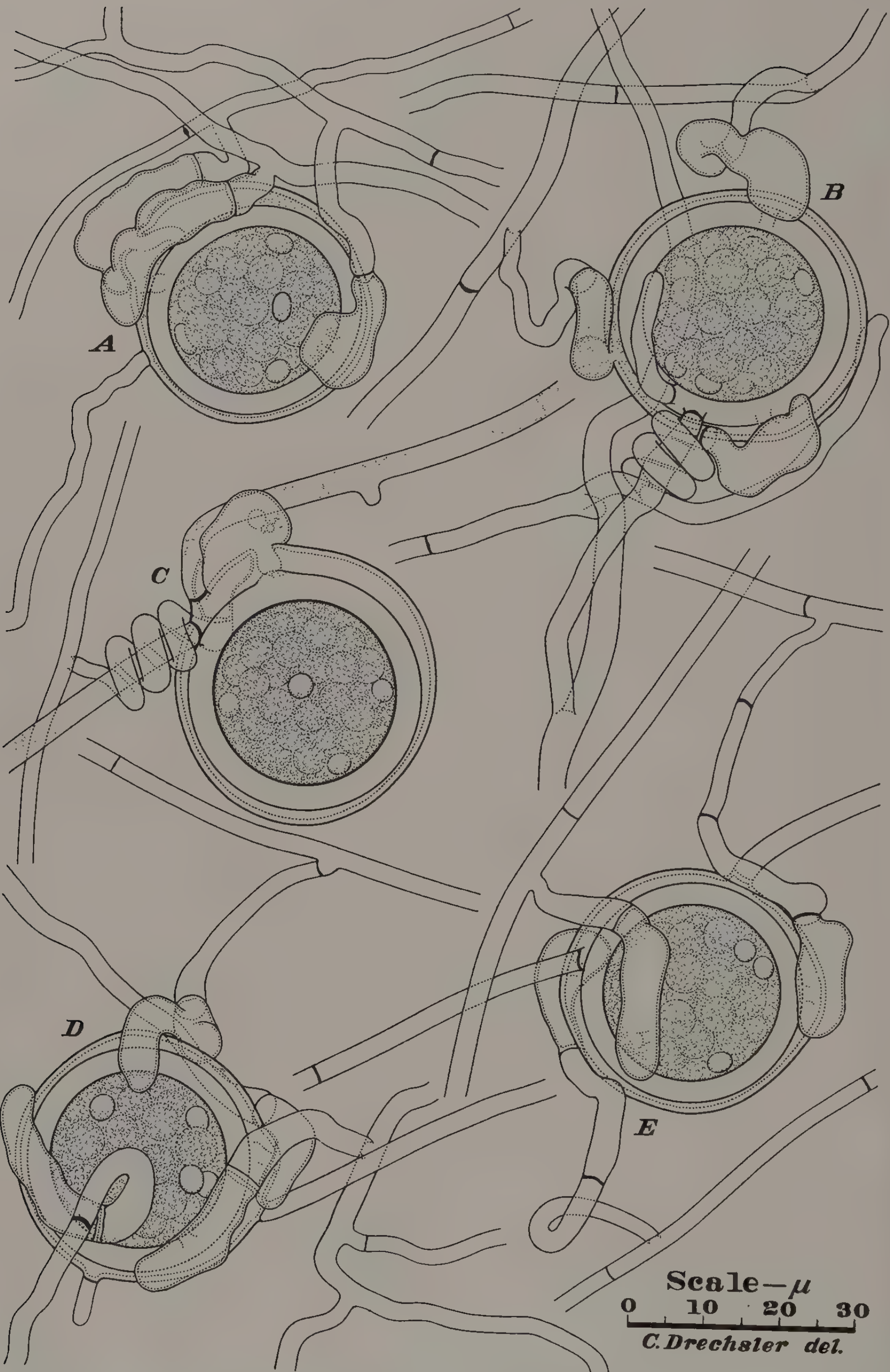
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FIG. 7. Sexual reproductive apparatus of *Pythium palingenes* produced in maizemeal agar, and drawn with aid of a camera lucida; ×1000.

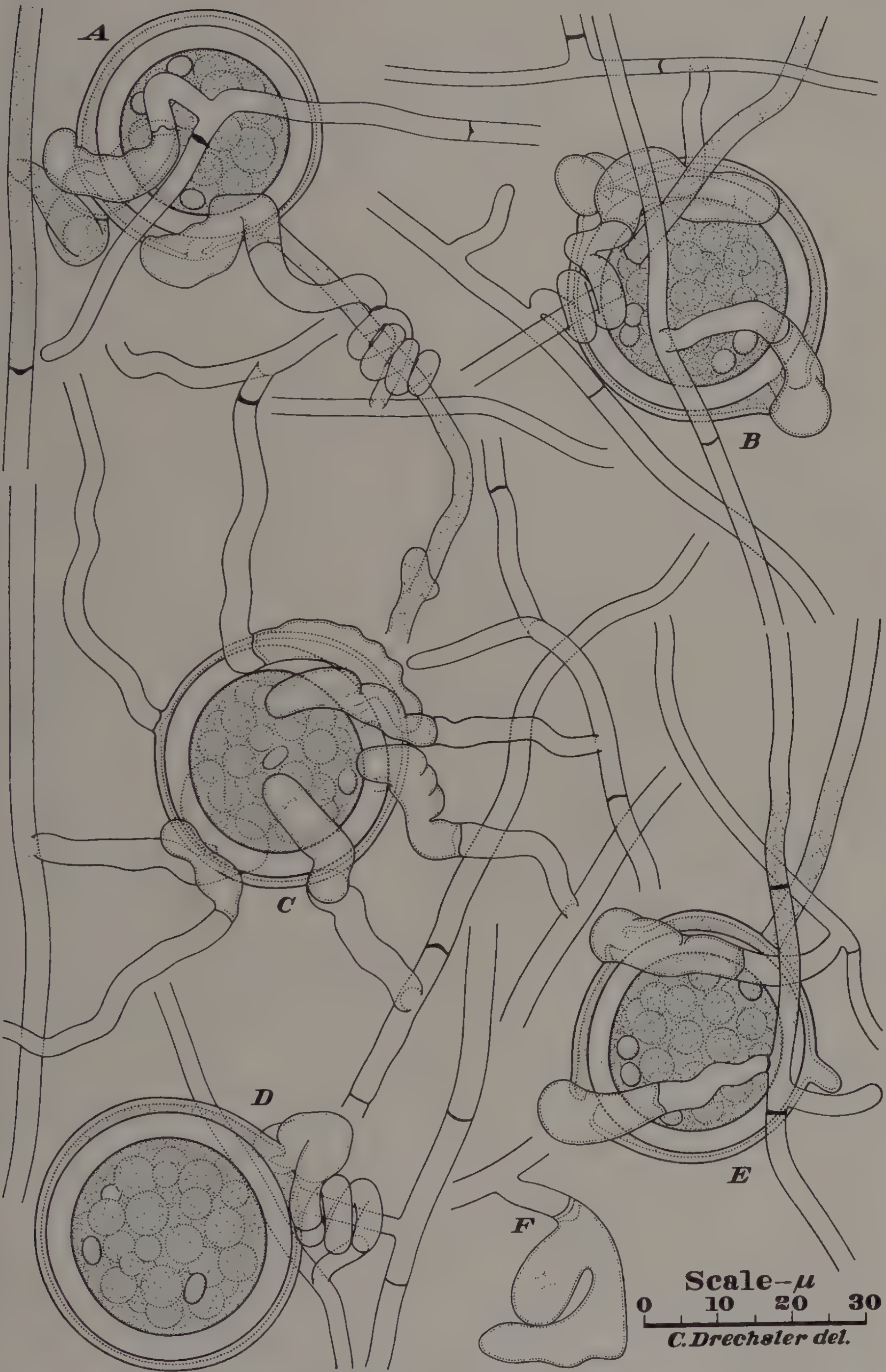
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FIG. 8. Sexual reproductive apparatus of *Pythium palingenes* produced in maizemeal agar, and drawn with the aid of a camera lucida; × 1000.

of variable lengths arising from hyphae having no close connection with the oogonial hypha. Although occasionally 6 (Fig. 8, C) and even 7 or 8 male organs have been found supplying an oogonium, the number of antheridia in units of sexual apparatus varies commonly from 1 to 4. Usually a proportion of sexual units varying from $\frac{1}{4}$ to $\frac{1}{2}$ show helicoid involvement of the oogonial hypha by an antheridial branch (Fig. 7, B, C; Fig. 8, B, D), or by a hyphal element closely connected with an antheridial branch (Fig. 8, A); the individual helical coil consisting ordinarily of 2 or 3 turns. As a rule the antheridia, which, like those of *P. helicoides* and *P. oedochilum,* are of an elongated cylindrical shape, though modified rather often by prominent dorsal undulation (Fig. 7, A; Fig. 8, C) or lobate branching (Fig. 8, F), are applied lengthwise to the oogonium. The fertilization tube usually arises from a position closer to the apex than to the base of the antheridium, and in occasional instances, where through irregularity of development the antheridium is applied only by its anterior end, may arise from a virtually apical position (Fig. 7, B).

The thick-walled, frequently yellowish oospores of *Pythium palingenes* have a multiplicate internal organization, since they reveal usually from 15 to 30 reserve globules and from 2 to 5 refringent bodies imbedded in a matrix of murkily granular protoplasm. Often the space between the oogonial membrane and the oospore wall is in large part filled with yellowish material, which sometimes appears to be of granular texture, or, again, may be of nearly homogeneous consistency (Fig. 7, A, B; Fig. 8, A, B, D, E). On the whole the variations in the main dimensions of oogonium and oospore may be considered moderate rather than extreme. As the values given in the original diagnosis for the diameters of these structures were based on sets of only 100 measurements, an equal number of additional measurements have since been made to make possible a metrical statement better comparable with metrical statements submitted for other species. An average of 33.6 μ was computed from the combined 200 values for oogonial diameter, which, expressed to the nearest integral number of microns, show a distribution as follows: 19 μ, 1; 20 μ, 1; 21 μ, 2; 23 μ, 3; 24 μ, 5; 25 μ, 2; 26 μ, 1; 27 μ, 1; 28 μ, 1; 29 μ, 4; 30 μ, 6; 31 μ, 10; 32 μ, 15; 33 μ, 24; 34 μ, 30; 35 μ, 25; 36 μ, 28; 37 μ, 15; 38 μ, 14; 39 μ, 7; 40 μ, 4; 41 μ, 1. And the combined 200 values for diameter of oospore, from which an average of 30.7 μ was computed, show a distribution as follows: 18 μ, 2; 19 μ, 2; 20 μ, 1; 21 μ, 4; 22 μ, 3; 23 μ, 3; 24 μ, 2; 26 μ, 3; 27 μ, 5; 28 μ, 17; 29 μ, 17; 30 μ, 17; 31 μ, 24; 32 μ, 30; 33 μ, 24; 34 μ, 20; 35 μ, 17; 36 μ, 7; 37 μ, 2. Through inclusion of the additional measurements the range in thickness of oospore wall was extended upward to a maximum of 3.8 μ, while the arithmetical mean of this dimension was increased to 2.8 μ.

A PROLIFEROUS SPECIES OF PYTHIUM WITH ASYMMETRICAL UTRIFORM SPORANGIA

Representatives of the genus *Pythium* were apparently first recorded as occurring naturally on materials of the water-lily family by Petersen (19) in statements relating to the habitats of *P. proliferum* de Bary and *P. undulatum* Pet. Working in Denmark, he observed the former species (or what he considered to be the former species) 3 times on the leaves and stems of old *Nymphaea* plants and on the decayed flower of a *Nuphar* plant; while the latter species of *Pythium* was encountered frequently on decaying leaves and stems of *Nuphar luteum* Sibth. and Smith and of *Nymphaea alba* Presl., though, occasionally, it was found also on old iris fruits and on submerged branches of trees. It seems not to have been Petersen's intention to attribute any pathological relationship to the 2 oomycetes, for, in discussing the biological circumstances attending the occurrence of submerse fungi, he expressed the view that aquatic plants—presumably aquatic types of the higher plants—are not attacked as long as they remain alive and are left undisturbed in their original site. Attack on plants partly dead he believed extended only to the dead parts.

From observations carried out during 2 seasons Dissmann (5) found that volunteer water-lily (*Nymphaea candida* Presl.) plants, growing abundantly in an artificial pond in marshy terrain near Hirschberg, Bohemia, showed yellowing of their leaves as early as May and June, although, ordinarily, foliar yellowing was not wont to occur until near the end of the growing season in autumn. The affected leaves soon turned brown and then became detached from the rhizome. On examining them with the naked eye they were found covered uniformly with a delicate filamentous nap, about 0.5 mm. in thickness, slimy to the touch. Microscopic inspection of this slimy nap disclosed a luxuriant hyphal growth and concomitant zoosporangia, which, from their manner of discharge, could for the most part be referred to the Pythiaceae. Sections of affected leaves showed the tissues to be thoroughly permeated with mycelial hyphae. Of the several oomycetes present in this material, 2 were consistently predominant. Studied in pure culture under varying conditions these 2 forms were identified with the 2 species previously recorded from water-lily materials by Peterson, namely, *Pythium proliferum* and *P. undulatum*. A relatively small proportion of affected leaves harbored, besides, 2 additional species of *Pythium*,—one of them from its production of spiny oogonia being held referable to *P. artotrogus* (Mont.) de Bary, whereas the other, with very minute oogonia, could not be identified with any species then described. The oldest portions of affected leaves, moreover, were regularly occupied by a saprolegniaceous form identified as *Achyla americana* Humph.

In our Northern States the foliage of some white water-lilies, including *Nymphaea odorata* Ait. and *N. tuberosa* Paine, often undergo premature decline from multiplication and extension of water-soaked or discolored areas. When portions of affected tissue are excised from such areas, pressed between sheets of absorbent paper to remove excessive water, and planted on agar media, *Pythium* mycelium almost always grows out very promptly. Mycelial growths thus obtained need only be freed of contaminating microorganisms to yield pure cultures. Among several hundred cultures derived through such procedure from material collected in Massachusetts, New York, and Wisconsin, a species closely similar to *P. helicoides,* and probably identical with Dissmann's *P. proliferum,* was abundantly represented; as was also a species that, judging from the large globose reproductive bodies produced by it in maize-meal agar, must almost certainly be the same as Dissmann's *P. undulatum.* Of less frequent occurrence, though present in more than a dozen cultures isolated from leaves of *N. tuberosa* collected near Butternut, Wis., in August 1938, was a species having an unusually distinctive asexual reproductive phase.

When grown on maize-meal agar the species in question extends its mycelium with a degree of rapidity moderate for members of the genus *Pythium.* The submerged mycelium produced in plate cultures is of a feebly lustrous, somewhat pronouncedly radiating appearance (Fig. 9, A). On microscopic examination it is seen to consist of relatively coarse axial hyphae, and relatively coarse branching systems; the more delicate ramifications in comparison with those of *P. periplocum,* for example, attaining only meager development. Swollen terminations, interpretable as appressoria, are produced very sparingly where the hyphal branches encounter the glass wall of a culture dish. Aerial mycelium ordinarily does not develop on maize-meal agar in Petri dishes, and often is likewise suppressed even in tube cultures.

As a general rule when the fungus is grown in pure culture on maize-meal agar or Lima-bean agar it gives rise to no zoosporangia whatever. Like other species of *Pythium,* it can be induced to reproduce asexually by transferring thin slabs of Lima-bean agar, well permeated with its mycelium, to a shallow layer of water. This treatment, however, usually results in only rather meager production of sporangia and in correspondingly meager development of zoospores. Yet, extraordinarily copious production of sporangia ensues without irrigation if several small pinches of leaf mold, or small quantities of other decaying vegetable detritus, are added to maize-meal agar cultures of the fungus; the efficacy of such amendment being especially marked if the medium employed is soft enough to afford a thin superficial film of free water. Often, within a few hours after the material has been added, zoosporangia will be found developing on the surface of the substratum in immediate proximity to the deposits of refuse. Their development and multiplication proceed actively for 5 to 10 days, spreading into more distant portions of the culture. However, the reproductive bodies are not distributed uniformly through the medium, being laid down for the most part in crowded arrangement along certain favored radials of the mycelium, mainly on the surface of the substratum, where they become visible collectively as flesh-colored radial streaks (Fig. 9, B).

On microscopic examination the more substantial of the streaky deposits are seen to consist of sporangia piled on one another several layers deep. Where the accumulations are somewhat less dense the individual sporangia

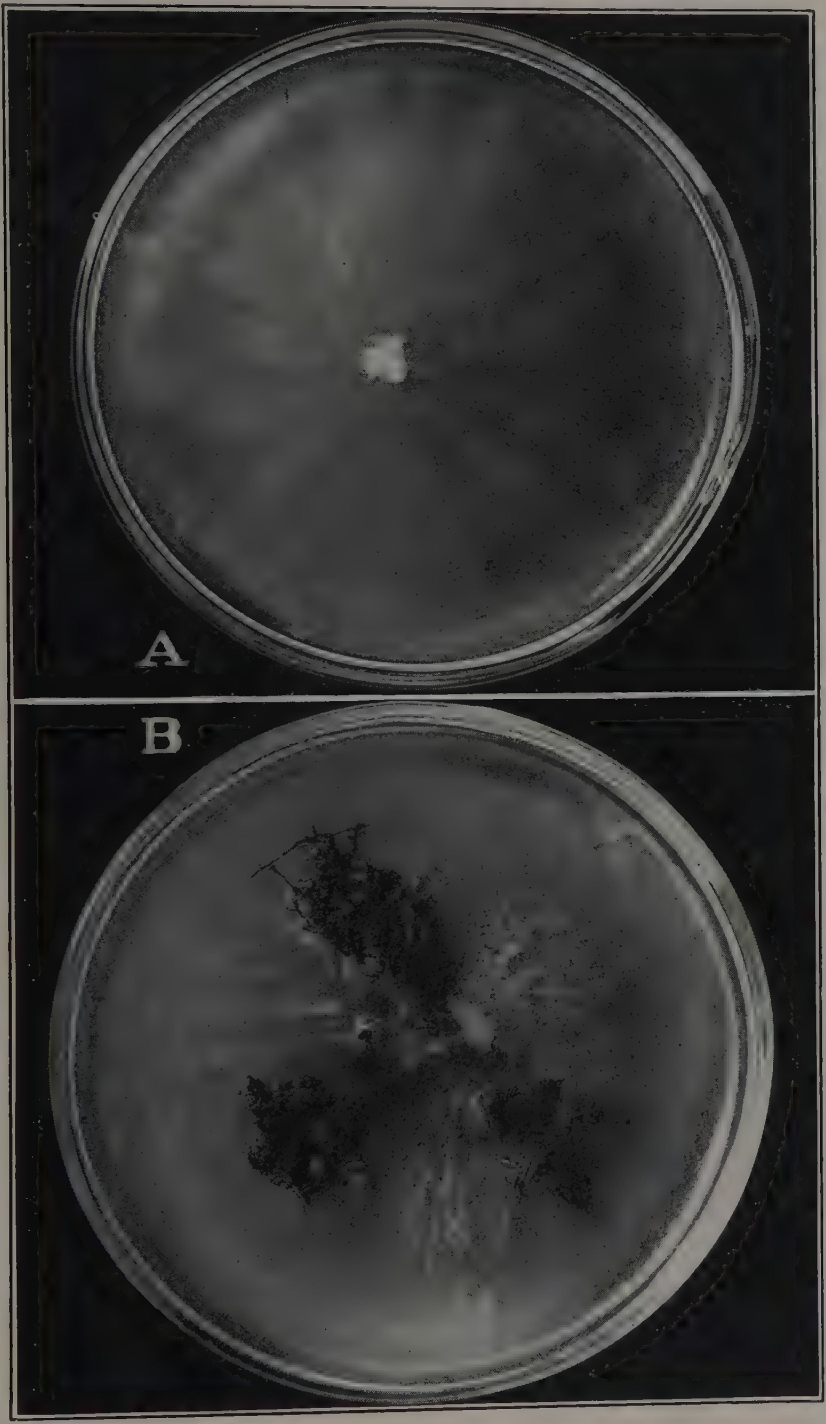

FIG. 9. Ten-day-old plate cultures of *Pythium marsipium* on maize-meal agar; natural size. A. No decaying plant material added,—sporangia absent. B. Leaf mold added after 4 days of growth,—innumerable sporangia present mainly in radial streaks.

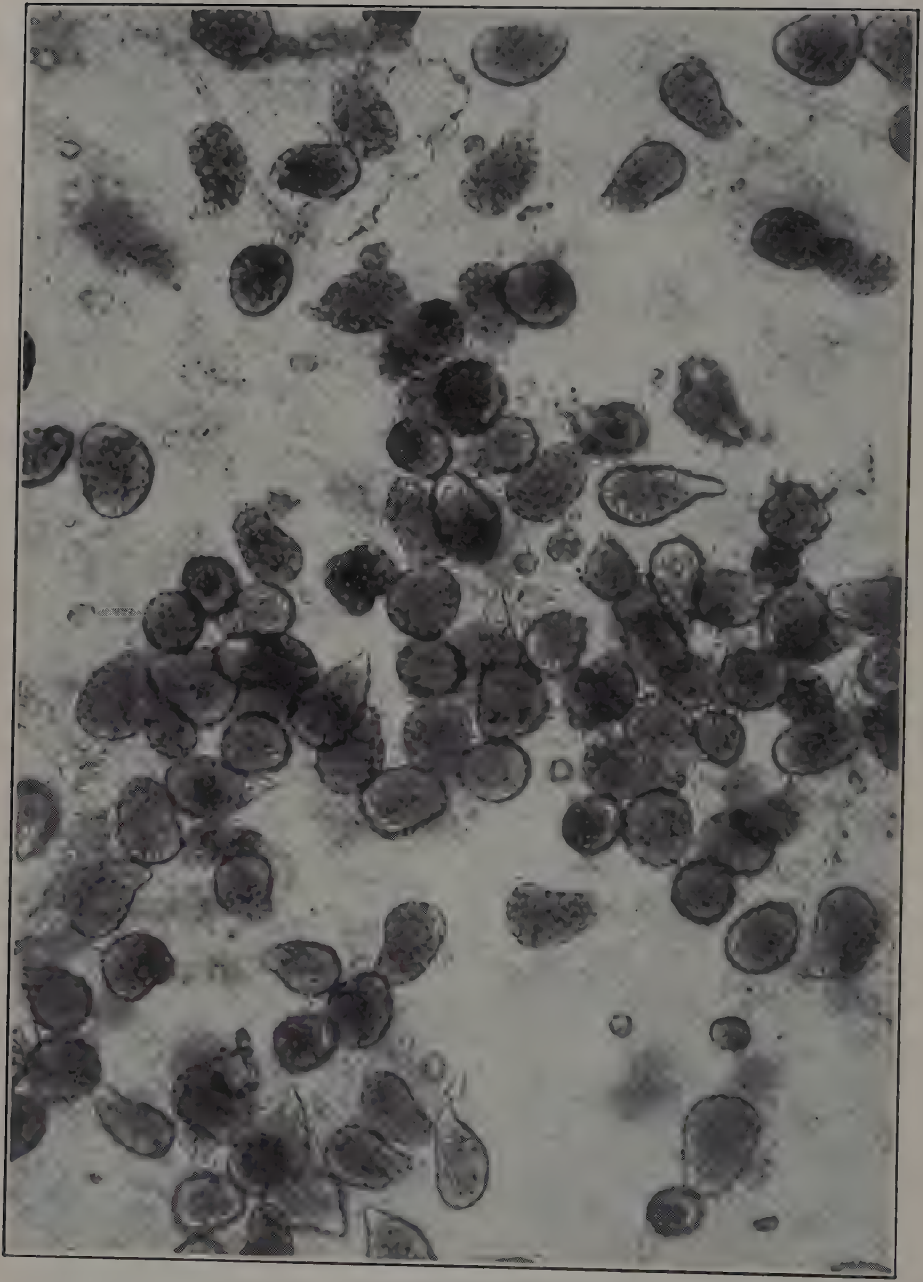

FIG. 10. Sporangia of *Pythium marsipium* in the plate culture shown in Fig. 9, B; about ×200.

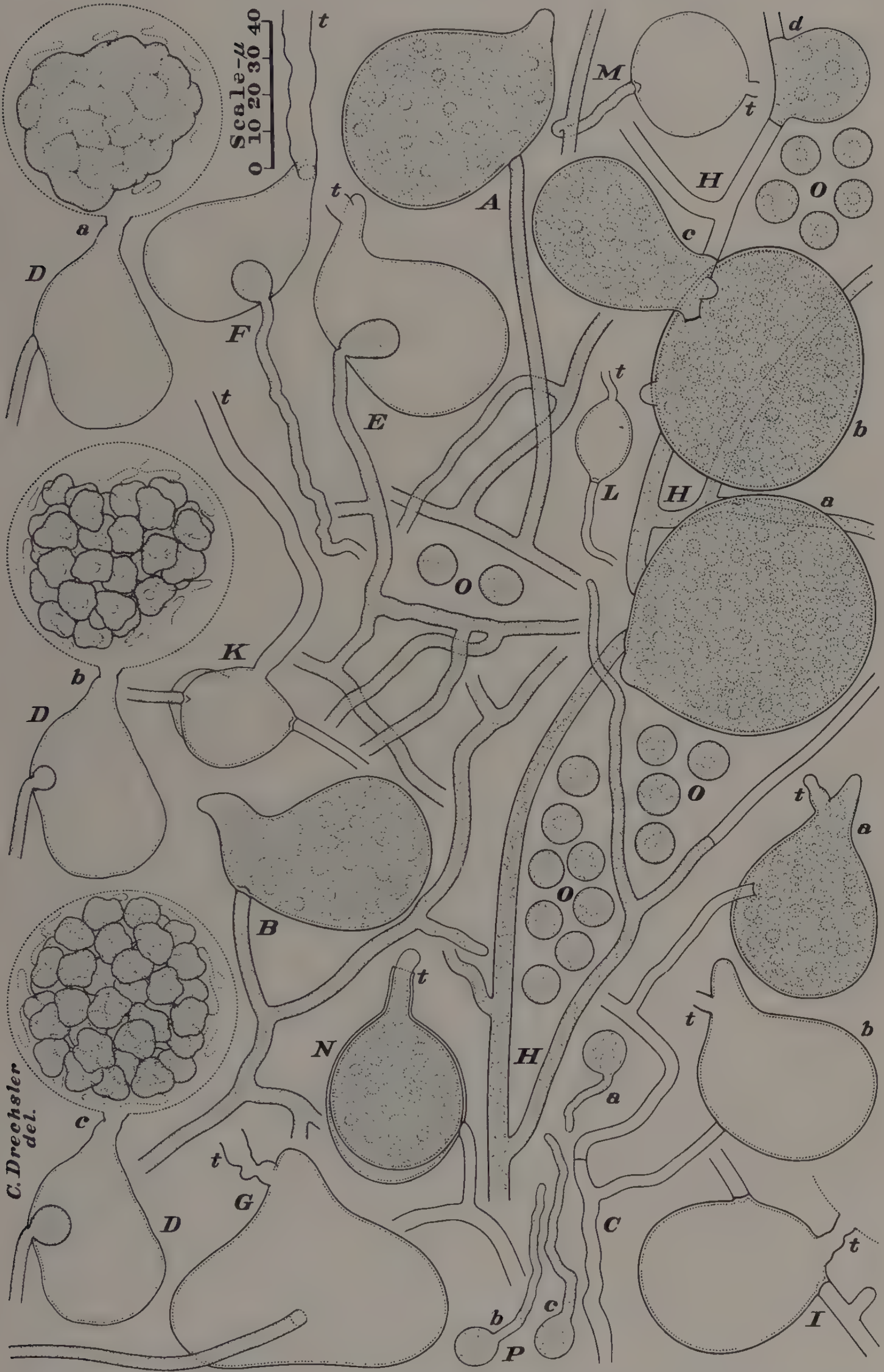
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FIG. 11. Asexual reproductive apparatus of *Pythium marsipium* originating in a maize-meal agar culture to which leaf mold had been added; drawn with aid of a camera lucida; ×500.

may be discerned as bodies of subspherical, ovoid, and saccate shapes (Fig. 10). A large proportion of the ovoid and saccate specimens are found to be attached laterally, so that their axes are directed athwart the axes of their supporting hyphae; the beak, moreover, being skewed rather sharply from the sporangial axis as if to prolong the supporting filament (Fig. 11, A, B). Comparable unsymmetry of shape, together with lateral attachment and transverse or oblique orientation, has long been known as a remarkable feature pertaining to sporangia of *Pythiogeton,* having been recorded for all 3 of the species—*P. utriforme, P. transversum,* and *P. ramosum*—described by von Minden (18) as initial members of the genus; though, it is true, the German investigator wisely decided not to recognize asymmetrical conformation of sporangia as a generic characteristic, after he had noted the presence in his material of spherical sporangia, as well as of sporangia intergrading between the spherical and the elongated asymmetrical types. Elongated asymmetrical sporangia I found to be produced also in my *P. autossytum* (7), again in association with spherical and intergrading specimens; while only subspherical and slightly ellipsoidal sporangia have been ascribed to *P. uniforme* Lund (16) and *P. dichotomum* Tokunaga (14), not to mention some unidentified congeneric species figured by Lund (16) and by Sparrow (22). Sporangia of regular ellipsoidal rather than utriform shape, yet with medio-lateral attachment and transverse orientation, have been set forth by Höhnk (12) in the description of his *Diasporangium Jonesianum.*

Although formation of zoospores in the fungus follows a course broadly similar to that usual for other species of *Pythium,* departures with respect to various details of development are observable. During the period immediately preceding discharge, the sporangium here does not reveal any vacuole comparable with the large lacuna-like vacuole that, in most congeneric forms, increases in size until it abruptly disappears very shortly before the protoplasmic materials are expelled. Internally the numerous sporangia in moist agar cultures amended with leaf mold often maintain without noticeable change for several days a granular structure the uniformity of which is relieved only by a more nearly homogeneous texture in the beak, and by the presence of numerous, more or less evenly distributed, subspherical hyaline bodies, approximately 3 μ in diameter. Perhaps more because of some lumping of protoplasm about these bodies than because of any definite demarcation, the sporangial contents present an appearance vaguely suggestive of cleavage, a little like that presented, for example, in sporangia of *Phytophthora* ready to discharge their zoospores. When water is added discharge of the sporangial contents ensues more promptly than in most allied species. Often within 30 minutes an evacuation tube is pushed out in proximity to the beak (Fig. 11, C, a), or the beak itself becomes elongated; a cap of dehiscence being formed in either case, which, on yielding, receives the escaping protoplasmic materials, and is thereby inflated into a spherical vesicle (Fig. 11, D, a). Almost immediately after its reception in the vesicle the discharged mass begins to show faint lines of cleavage, and to display waving cilia at its periphery. Manifestly, at the time of their discharge, the sporangial contents here have already undergone the initial stages of reorganization that elsewhere are accomplished during the first 5 minutes of their sojourn within the vesicle. Since the further stages of transformation follow in the sequence usual for species of *Pythium*, the laterally biciliate zoospores are ready to be liberated after 15 to 20 minutes, rather than after 20 to 25 minutes as in most congeneric forms. The peculiarities attending asexual reproduction in the genus *Pythiogeton*—discharge of sporangial contents into a conspicuously elongated vesicle, deliquescense of the vesicular envelope either in place or after violent projection of the extruded mass, and fashioning of zoospores from the unprotected protoplasm—have not been observed in the fungus.

The distinctive asymmetrically beaked sporangia (Fig. 11, C, b; E; F), as also the occasional bilobed specimens (Fig. 11, G) of apparently binate origin, are for the most part borne terminally on mycelial branches. In very prolific agar cultures a large proportion of the sporangia arise as intercalary bodies, often close together, on a main hypha (Fig. 11, H, a–d). These, more usually, are subspherical or ellipsoidal, and as a rule bear a sessile papilla (Fig. 11, H, a, b, c). The papilla, though sometimes functioning in dehiscence without further~~ing~~ elongation, more frequently grows out into an evacuation tube of varying length (Fig. 11, I, t; K, t). Among the globose intercalary sporangia may be found specimens as much as 60 or 70 μ in diameter, which, on discharging, give rise to more than a hundred zoospores. Of course not all globose or ellipsoidal sporangia are intercalary, some being borne terminally (Fig. 11, L, M) much like asymmetrical specimens.

After a sporangium has been evacuated, the supporting hypha may resume growth by giving rise, usually within the emptied envelope, to a second sporangium (Fig. 11, D, b, c; E; F; N). Such proliferous development would appear, on the whole, somewhat more frequent in rather well-irrigated material than in material only sparingly irrigated. Deficiency in supply of water may often fail to inhibit sporangial discharge, even while it is causing premature disintegration of vesicles, with the result that numerous discharged protoplasmic masses, deprived of their protective coverings, undergo degeneration on a relatively enormous scale. Under proper conditions, however, irrigation of slabs excised from cultures on maize-meal agar, amended with leaf mold, leads within 1 or 1.5 hours to liberation of active zoospores in extraordinary abundance. After swimming about for some time the zoospores round up (Fig. 11, O), to germinate later by the production usually of a single germ tube (Fig. 11, P, a–c).

In its sexual reproduction the fungus is somewhat capricious. Most cultures on maize-meal agar show no evidence of sexual development, and, apparently, such development cannot be induced by increasing the amount of finely divided maizemeal in the medium, or by adding either leaf mold or yeast extract. Yet, now and then a culture on the same substratum will afford prompt production of oospores wholly correct in structure, without

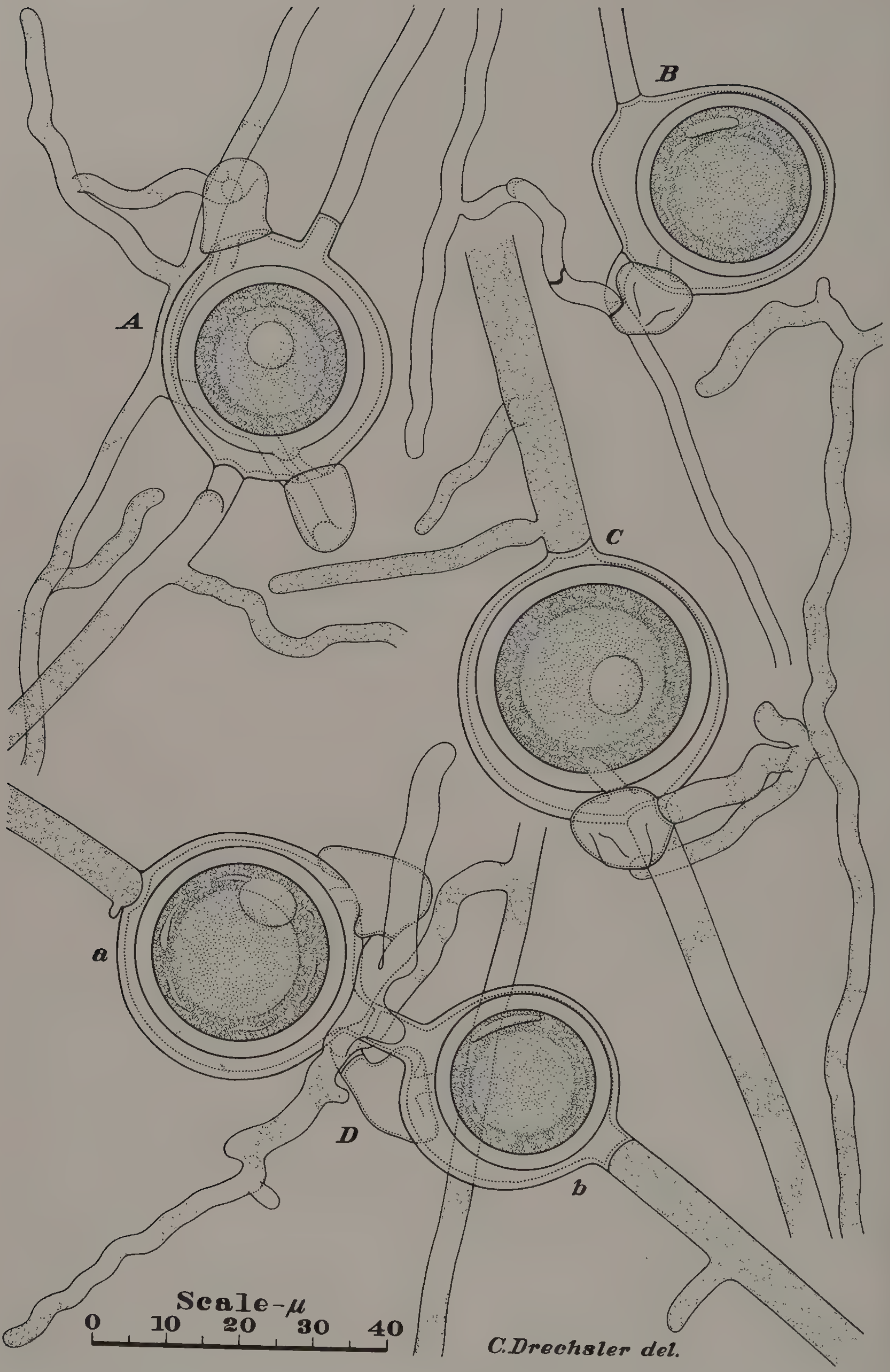
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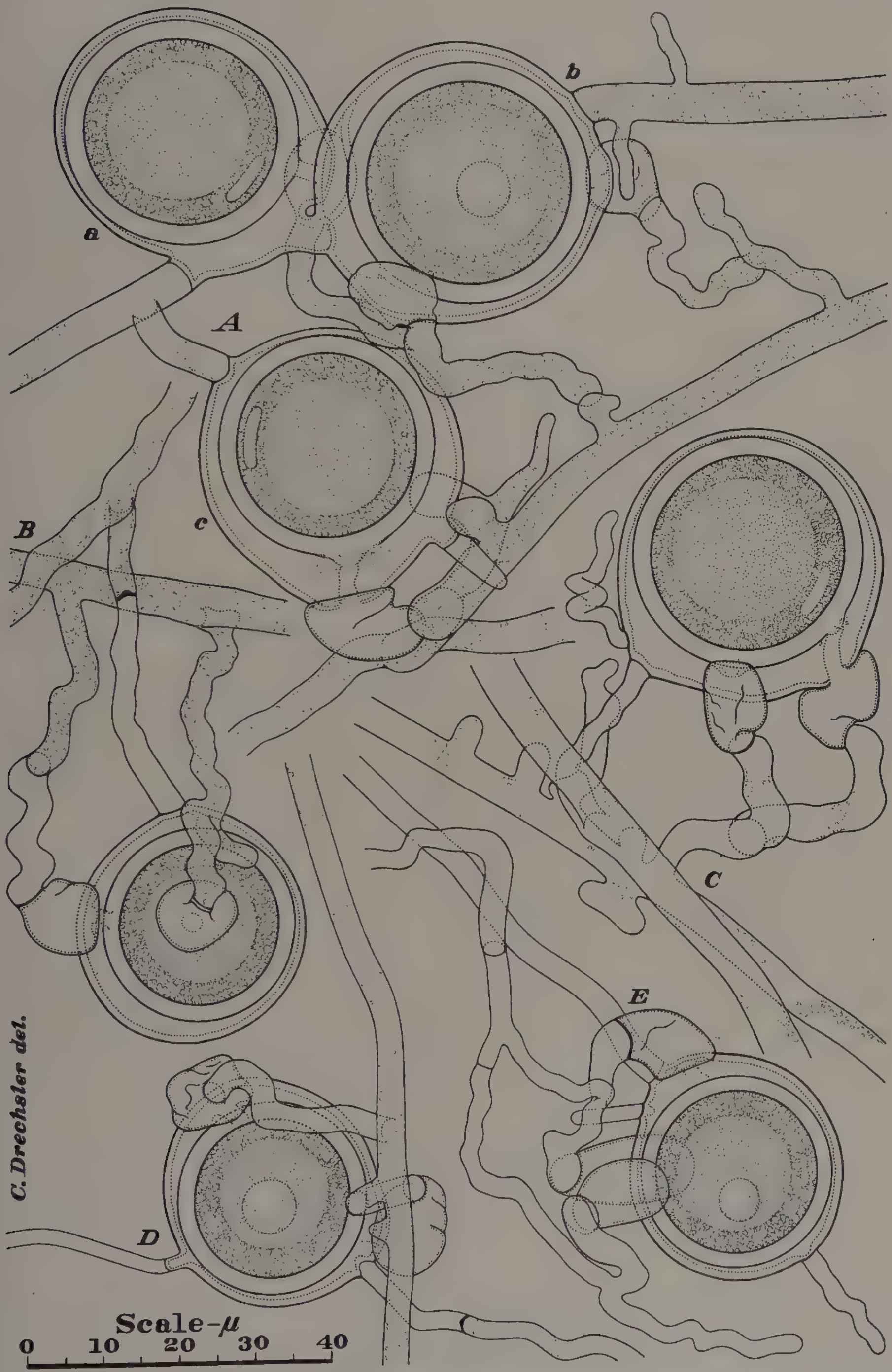
FIG. 12. Sexual apparatus of *Pythium marsipium* produced in maize-meal agar, and drawn with the aid of a camera lucida; ×1000.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A
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B
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E
C. Drechsler del.
Scale-μ
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30
40

FIG. 13. Sexual apparatus of *Pythium marsipium* produced in maize-meal agar, and drawn with the aid of a camera lucida; ×1000.

any addition of alien substances. Like the sporangia the units of sexual apparatus usually are found in crowded arrangement along favored hyphae, and thus collectively become visible to the naked eye in radial streaks.

The oogonia are rather large subspherical bodies, frequently intercalary in the wider mycelial filaments, either singly (Fig. 12, A; B; C; Fig. 13, D) or in pairs (Fig. 12, D, a, b; Fig. 13, A, a, b). Often, again, they are borne terminally (Fig. 13, B) or somewhat subterminally (Fig. 13, A, c; C; E) on branches of variable lengths. Fertilization is accomplished usually by 1, 2 or 3 antheridia, borne terminally on rather stout lateral branches arising from hyphae having no close connection with the oogonial filaments. Diclinism is conspicuous in the numerous aggregations of sexual apparatus where all the female elements clearly come from one hyphal system, and all male elements from another. In some instances an antheridium is brought into a lateral position when the supporting hypha continues growth from below the delimiting septum to produce a second antheridium farther onward. As a rule the antheridia are broadly applied to the oogonium, which often protrudes noticeably in the region of contact, besides showing here marked attenuation of its relatively thick envelope. The fertilization tube is often rather wide, and frequently may be seen attached by its distal end to a wart-like excrescence on the oospore wall (Fig. 12, A; Fig. 13, A, c; C). Internally, the mature oospore always reveals the unitary structure common to most species of *Pythium*, never the multiplicate structure distinctive of the *helicoides* series. Its single reserve globule is of comparatively large size, as is evident from the relevant metrical data given in the diagnosis, which were derived from measurements of 200 units of sexual apparatus selected at random in maize-meal agar cultures. Expressed to the nearest integral number of microns, the values for diameter of oogonium obtained from these measurements are distributable as follows: 23 μ, 1; 24 μ, 2; 25 μ, 3; 26 μ, 8; 27 μ, 13; 28 μ, 20; 29 μ, 17; 30 μ, 27; 31 μ, 23; 32 μ, 22; 33 μ, 18; 34 μ, 14; 35 μ, 10; 36 μ, 13; 37 μ, 7; 38 μ, 2; while the values for diameter of oospore are distributed thus: 19 μ, 1; 21 μ, 4; 22 μ, 9; 23 μ, 19; 24 μ, 20; 25 μ, 25; 26 μ, 33; 27 μ, 28; 28 μ, 13; 29 μ, 23; 30 μ, 11; 31 μ, 10; 32 μ, 2; 33 μ, 2.

Although the fungus produces asymmetrical utriform sporangia conforming to a type held characteristic more especially of several species of *Pythiogeton,* it clearly does not belong in that genus. As has been noted, the vesicle wherein the sporangial contents are fashioned into zoospores is consistently spherical, and not only fails to deliquesce soon after its inflation, but often shows more than ordinary durability when sizeable portions of its membrane persist in visible state for an hour or two after escape of the zoospores (Fig. 11, I). Despite some aberrant features—prolonged maintenance of the sporangia in a condition of readiness for prompt discharge, initiation of zoospore development previous to discharge—asexual reproduction here is unmistakably that of a species of *Pythium.* While pronounced vacuolization is not apparent in the sporangium shortly before dehiscence, it remains possible that the protoplasmic reorganization associated with such vacuolization may be accomplished here much less obtrusively just before the sporangium enters on its prolonged condition of readiness for discharge, that is, at a stage often long preceding discharge as regards time, yet, corresponding approximately to the developmental stage immediately preceding discharge in the generality of congeneric forms. On aging, naturally, the sporangia here, like those of other oomycetes, sooner or later show a vacuole of senescence, which often increases in size until the granular protoplasm is crowded into a thin parietal layer.

The literature on *Pythium* is not wholly free of comment in which fungi with unsymmetrical zoosporangia have been designated as species of that genus. Cornu (4), in 1872, made reference, under the binomial *Pythium utriforme,* to a new species whose zoosporangia he described as being always irregular, utriform, and elongate-reniform in shape; his further characterization of them as being occasionally intercalary presumably implying that like the spherical sporangia of another new species, *Pythium imperfectum,* they were borne for the most part terminally on delicate filaments similar to the sporangiferous filaments of *Pythium proliferum.* In the 2 new species, both proliferous like *Pythium proliferum,* the protoplasm issued from the tip of a long evacuation tube, to be received at the beginning in a vesicle, which, however, normally burst after attaining very meager development. Von Minden properly recognized the external shape ascribed to the sporangium of *Pythium utriforme,* and the early disintegration, in that species, of the vesicular membrane, as features indicating probable identity with one or another of the 3 species he described in the genus *Pythiogeton.* Though he believed, not without justification, that *Pythium imperfectum* was precluded from such identity by reason of its spherical sporangia, this inadequately described species, with its early disintegration of vesicular envelope, would now seem referable to *Pythiogeton* no less properly than *Pythiogeton uniforme, Pythiogeton dichotomum,* and the several unnamed congeneric forms figured by Lund and Sparrow, for all of which likewise only globose sporangia have become known.

Soon after Cornu's insufficient characterization of *Pythium utriforme,* Pringsheim (20) referred somewhat incidentally to a closely allied species, which, from the varied, mostly lateral origin of its evacuation tube, he designated as *Pythium laterale.* This species, though inviting von Minden's speculations relative to its possible affinities in *Pythiogeton,* could only be dismissed from consideration, since the relevant descriptive comment, again, gave no clear picture of the fungus observed.

From the several remaining proliferous forms that have been described as species of *Pythium* the fungus would seem to differ primarily in its production of utriform sporangia. Apart from this general difference, the habitual declinism of its sexual phase offers a marked contrast with *P. proliferum,* in which, according to de Bary (1), the antheridium as a rule arises from the oogonial hypha in close proximity to the oogonium, and only occasionally is contributed by a neighboring filament. Later writers that dealt at first hand with some proliferous form to which they applied de Bary's familiar binomial have not always, it must be admitted, insisted on predominance of the monoclinous relationship. Of the 3 antheridia shown in Ward's figures (23, pl. 35, fig. 20, 21) one is clearly monoclinous, one is diclinous, and the other is of ambiguous origin. Butler (3) found male organs coming from neighboring hyphae more frequently than from the oogonial hypha. In Matthews' (17) account the antheridia are figured and described as coming rather impartially from neighboring filaments or from the oogonial filament, in the latter instance arising either adjacent to the oogonium or at some distance from it.

In his original account of *Pythium ferax,* de Bary (2) characterized this form as being very similar to *P. proliferum,* though differing in various details. Among the details representative of difference was mentioned the absence of a large vacuole in the sporangium. As stalk antheridia, that is, antheridia made up individually of a segment of oogonial hypha adjacent to the oogonium, were stated to predominate over branch antheridia the sexual apparatus of *P. ferax* presents an arrangement of parts wholly alien to the present fungus.

Dissimilarity with respect to origin of antheridia likewise sets the fungus apart from *Pythium nagai* Ito et Tokunaga (13), a proliferous species associated with decay of rice (*Oryza sativa* L.) seedlings in Japan. Here, oogonia measuring only 14 to 22 μ in diameter are fertilized usually by a single male organ, ovoid, globose, or clavate in shape, that is borne terminally on a slender branch arising from the oogonial stalk.

Ito (14) in his diagnosis of *Pythium iwayamai,* a species found by Iwayama (15) to attack barley (*Hordeum sativum* Jess.) and wheat (*Triticum vulgare* L.) in Japan, makes no mention of a proliferous habit, though the terminal attachment of sporangia described in part as being spherical, or more rarely ellipsoid, ovoid, or lemon-shaped, offers some obvious parallelism with proliferous forms. The oospores of this parasite, which measure 19 to 24 μ in diameter, are not only smaller than those of the fungus under consideration but differ further in that they completely fill the oogonial chamber.

Parallelism with proliferous forms is suggested also in the subspherical or lemon-shaped conformation attributed by Sideris (21) to the sporangia of 2 fungi he described as new species under the names *Pythium polycladon* and *P. euthyhyphon,* after he had isolated them in Hawaii from castor bean (*Ricinus communis* L.) roots and pineapple (*Ananas sativus* Schult.) roots, respectively. To these species were ascribed, somewhat misgivingly, oogonia and oospores imperfectly seen within host tissue. The measurements given for the diameters of the bodies observed—about 20 μ for the bodies presumed to belong to *P. polycladon,* and about 18 μ for those referred to *P. euthyhyphon*—are well below the usual values for any analogous dimension in my
sporangia, which often show proliferous development. In an old culture on
fungus.

Pythium carolinianum Matthews (17), described as a parasite on a species of *Spirogyra* in North Carolina, gives rise to subspherical or elongated carrots, some of its sporangia were observed to form bodies resembling a young oospore in appearance but never developing typical oospore structure. These bodies, dubiously designated as resting spores, were figured with a lumpy internal organization slightly suggestive of the multiplicate organization in normal, mature oospores of *P. helicoides* and its allies. As its hyphae were stated to be only 1 to 4 μ wide, *P. carolinianum* would seem appreciably more delicate than any proliferous congeneric form I have encountered.

In fine, the fungus from water-lily leaves appears not to have been previously described, and is, therefore, presented as a new species; the specific name applied to it—a term meaning "small pouch"—having reference to its bursiform sporangia.

Pythium marsipium, sp. nov.

Mycelium hyalinum, ramosum, in hyphis 2–7.5 μ crassis consistens. Zoosporangiis modo sphaeroideis, papilla instructis, 20–70 μ diam., modo terminalibus, elongato-ovoideis vel utriformibus, saepius 25–70 μ longis et 20–45 μ latis, basi rotundatis, apice oblique rostratis, a latere transverse vel oblique ramo saepe 20–100 μ longo et 3–4 μ crasso applicatis; subinde per papillam vel per rostrum exinanientibus, sed saepius per tubulum usque 100 μ longum 2–8 μ crassum protoplasma eorum fundentibus. Zoosporis saepius 25–75 in vesicula evolutis, reniformibus, a latere biciliatis, quiescentibus globosis, 9–12 μ diam. Oogoniis terminalibus, subterminalibus, vel intercalaribus, globosis, 23–39 μ (saepe circa 30.9 μ) diam., membrana .7–1.2 μ crassa tectis; antheridiis interdum singulis, plerumque binis ternisve, rarius quaternis, fere terminalibus, saepissime diclinis, cupulatis vel campaniformibus, 10–20 μ longis, 8–12 μ latis, apice ad oogonium late appositis; oosporis flavidis, sphaericis, 19–33 μ (saepe circa 26.2 μ) diam., membrana 1.3–2.8 μ (saepe circa 1.95 μ) crassa tectis, unam pilulam oleosam 11–22 μ (saepe circa 16.6 μ) diam. et unum corpusculum nitidum globosum vel valde applanatum continentibus.

Habitat in foliis Nymphaeae tuberosae putrescentibus prope Butternut, Wisconsin.

Intramatrical mycelium in transparent agar media often slightly lustrous, of pronouncedly radiated appearance, capable of approximately 19 μ radial extension in 24 hours at 24° C., composed of hyphae 2 to 7.5 μ wide, the more delicate ramifications being present usually only in moderate quantity. Aerial mycelium often absent or rather sparingly developed; under aquatic conditions extramatrical growth rather scanty.

Sporangia subspherical or often unsymmetrically utriform,—the subspherical specimens mostly 20 to 70 μ in diameter, papillate, produced mainly under moist rather than aquatic conditions, sometimes terminal but more often intercalary in wide filaments,—the utriform specimens measuring 25 to 70 μ in length by 20 to 45 μ in width, broadly rounded at one end and skewly beaked at the other, formed under moist or under aquatic conditions for the most part terminally on branches often 20 to 100 μ long and 3 to 4 μ wide, their long axis transverse or oblique to the supporting filament; discharging sometimes by means of the beak or papilla without elongation of these parts, but more often by means of an evacuation tube, 2 to 8 μ wide and up to 100 μ long, which may prolong the beak or papilla, or may arise from a position nearby; proliferous in variable measure, usually by the production of a secondary sporangium within the empty envelope of a primary one. Zoospores bean-shaped, longitudinally grooved, laterally biciliate, produced within a moderately durable subspherical vesicle in numbers ranging from 2 to 125, usually from 25 to 75; after rounding up measuring mostly 9 to 12 μ in diameter.

Oogonia frequently terminal or subterminal on sturdy branches, but more often intercalary in the coarser axial hyphae, and then sometimes occurring adjacent to one another; subspherical, though often protruding noticeably toward an applied antheridium; measuring 23 to 39 μ (average 30.9 μ) in diameter; provided individually with a sturdy wall .7 to 1.2 μ in thickness; and supplied with 1 to 4, usually 1 to 3, antheridia. Antheridia usually borne terminally on stout branches arising from filaments not closely connected with the oogonial hypha, though sometimes coming into a lateral position through prolongation of the supporting branch; irregularly expanded, prolate ellipsoidal, cupulate, or bell-shaped; measuring mostly 10 to 20 μ in length by 8 to 12 μ in width; at the distal end mostly rather broadly applied to the oogonium; intruding a fertilization tube often 2 to 5 μ long and 1.5 to 2.5 μ wide. Oospores usually yellowish, subspherical; measuring mostly 19 to 33 μ (average 26.2 μ) in diameter; provided with a wall 1.3 to 2.8 μ (average 1.95 μ) in thickness; containing a single spherical reserve globule 11 to 22 μ (average 16.6 μ) in diameter; and having imbedded in the narrow parietal granular layer a single refringent body which measures about 5 μ in diameter when of subspherical shape, but which is often strongly flattened into an oblate ellipsoid and then measures 1.3 to 2.5 μ in thickness by 7 to 10 μ in width.

Occurring in discolored, watersoaked, or decaying leaves of *Nymphaea tuberosa* near Butternut, Wis.

SUMMARY

Pythium oedochilum and *P. palingenes* resemble *P. helicoides* in the generally symmetrical conformation and proliferous development of their sporangia, which, however, owing to infrequence of distal branching in the supporting hyphae, are rarely borne in corymbose arrangement. Asexual reproduction in *P. palingenes* is often characterized by abundant iterative swarming; the repetitional development coming about both through direct evacuation of the cyst envelope, and through production of a miniature sporangium on a germ sporangiophore. The sexual reproduction of *P. oedochilum* and *P. palingenes* shows parallelism with that of *P. helicoides* not only in the longitudinal application of elongated antheridia to oogonia that often protrude along the regions of contact, but also in the distinctively multiplicate internal structure of the ripe oospore,—this spore containing plural reserve globules and plural refringent bodies instead of the single reserve globule and single refringent body found in the oospores of most species of *Pythium*. Helicoid involvement of the oogonial hypha by an antheridial branch, such as is associated with every unit of sexual apparatus in *P. helicoides*, occurs very rarely in *P. oedochilum*, and is concomitant with about one-third of the sexual units in *P. palingenes*.

A fungus isolated from affected portions of water-lily leaves is described as a new species under the name *Pythium marsipium*. In agar cultures its asexual reproduction is enormously increased by addition of various decaying plant materials. Apart from zoosporangia of commonplace subspherical shape, often intercalary in the longer mycelial filaments, it produces, for the most part terminally on fairly short branches, asymmetrical utriform zoosporangia of a type that in recent times has come to be considered more or less distinctive of the genus *Pythiogeton*. Its zoospores are fashioned within a spherical vesicle of at least ordinary durability, from a mass of protoplasm, which, previous to sporangial dehiscence, has usually undergone the preliminary changes that in most congeneric forms are accomplished during the first 5 minutes following dehiscence. Proliferous development of sporangia occurs under thoroughly aquatic conditions. As the fungus produces oospores of unitary internal structure—that is, oospores with a single reserve globule and single refringent body—it must be regarded as alien to the *helicoides* series.

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LITERATURE CITED

1. BARY, A. DE. Untersuchungen über die Peronosporeen und Saprolegnieen und die Grundlagen eines natürlichen Systems der Pilze. Abhandl. Senckenb. Naturf. Gesell. **12**: 225–370. 1881.
2. ———. Zur Kenntnis der Peronosporeen. Bot. Ztg. **39**: 521–530, 537–544, 553–563, 569–578, 585–595, 601–609, 617–625. 1881.
3. BUTLER, E. J. An account of the genus *Pythium* and some *Chytridiaceae*. India Dept. Agr. Mem., Bot. Ser. **1**: no. 5. 1907.
4. CORNU, M. Monographie des Saprolégniées, étude physiologique et systématique. Ann. Sci. Nat. Bot. Sér. 5, **15**: 5–198. 1872.
5. DISSMANN, E. Vergleichende Studien zur Biologie und Systematik zweier *Pythium*-Arten. Arch. Protistenk. **60**: 142–192. 1927.
6. DRECHSLER, C. Some new species of *Pythium*. Jour. Washington Acad. Sci. **20**: 398–418. 1930.
7. ———. A species of *Pythiogeton* isolated from decaying leaf-sheaths of the common cat-tail. Jour. Washington Acad. Sci. **22**: 421–449. 1932.
8. ———. Two hyphomycetes parasitic on oospores of root-rotting oomycetes. Phytopath. **28**: 81–103. 1938.
9. ———. Several species of *Pythium* causing blossom-end rot of watermelons. Phytopath. **29**: 391–422. 1939.
10. ———. Three species of *Pythium* with large oogonial protuberances. Phytopath. **29**: 1005–1031. 1939.
11. ———. Three species of *Pythium* associated with root rots. Phytopath. **30**: 189–213. 1940.
12. HÖHNK, W. On three pythiaceous oomycetes. Beih. Bot. Centralbl. **55** (Abt. A): 89–99. 1936.
13. ITO, S., and Y. TOKUNAGA. Studies on the rot-disease of rice-seedlings caused by *Pythium*-species. Jour. Fac. Agr. Hokkaido Imp. Univ. Sapporo, Japan. **32**: 201–228. 1933.
14. ———, and ———. Notae mycologicae Asiae orientalis I. Trans. Sapporo Nat. Hist. Soc. **14**: 11–33. 1935.
15. IWAYAMA, S. On a new snow-rot disease of cereal plants caused by *Pythium* sp. Toyama Agr. Exp. Stat. Publ. 20 pp. 1933.
16. LUND, A. Studies on Danish freshwater phycomycetes and notes on their occurrence particularly relative to the hydrogen ion concentration of the water. Danske Vidensk. Selsk. Skr. Afd. **6**: 3–98. 1934.
17. MATTHEWS, VELMA D. Studies on the genus *Pythium*. 136 pp. The University of North Carolina Press (Chapel Hill). 1931.
18. MINDEN, M. VON. Beiträge zur Biologie und Systematik einheimischer submerser Phycomyceten. Mykologische Untersuchungen und Berichte von Dr. Richard Falck **1**: 146–255. 1916.
19. PETERSEN, H. E. An account of Danish freshwater-phycomycetes, with biological and systemical remarks. Annales Mycologici **8**: 494–560. 1910.
20. PRINGSHEIM, N. Weitere Nachträge zur Morphologie und Systematik der Saprolegnieen. Jahrb. Wiss. Bot. **9**: 191–234. 1873–1874.
21. SIDERIS, C. P. Taxonomic studies in the family Pythiaceae. II. *Pythium*. Mycologia **24**: 14–61. 1932.
22. SPARROW, F. K. A contribution to our knowledge of the aquatic phycomycetes of Great Britain. Jour. Linnean Soc. (London) **50**: 417–478. 1936.
23. WARD, H. M. Observations on the genus *Pythium* (Pringsh.). Quart. Jour. Micros. Sci. (London) (n.s.) **23**: 485–515. 1883.

TWO PHYSIOLOGIC RACES OF HELMINTHOSPORIUM MAYDIS IN THE CORN BELT[1]

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INTRODUCTION

In 1938, a severe disease caused by a species of *Helminthosporium* was observed in Indiana on the dent corn (*Zea mays* L.) inbred "Pr". All aerial parts of the plants were attacked and in many instances nearly a total loss resulted. The complete involvement of the plants caused premature killing and severe rotting of the ears. The disease occurred with equal severity again in 1939.

In 1939, a disease, exhibiting strikingly different symptoms, was observed in the field on inbred "187–2" and on a number of experimental and proprietary lines. Further study demonstrated that the parasite involved is morphologically indistinguishable from the fungus isolated from inbred Pr. Cross-inoculation tests in the greenhouse on seedlings of various inbreds have shown, however, that constant differences in symptoms exist that are not due primarily to response of the host. These tests thus far have shown that, among the inbreds tested, Pr is the only host of the fungus isolated from it, and that the parasite isolated from 187–2 and other lines has a wider host range so far as inbreds and hybrids of corn are concerned.

This paper reports studies conducted on two groups of isolates of a species of the genus *Helminthosporium* that are morphologically alike, but differ markedly in the symptoms they produce, and in the lines of corn they parasitize.

COMPARATIVE MORPHOLOGY

Diseased leaves of inbred Pr and of a proprietary inbred were collected in the field and placed in separate moist chambers for five days. Observations on size, shape, and number of septa were made of 100 conidia selected at random from each of the specimens. No significant differences in mean spore length, mean spore width, number of septa, or spore shape were found between the two samples. Conidiophores from the two specimens exhibited no morphological differences. When grown on various agar substrates the colonies of the two isolates could not be distinguished on the basis of cultural behavior.

Collection of diseased materials from various localities was continued as the season progressed. Eight specimens of inbred Pr consisting of diseased leaves or ears, and 8 from other inbreds were each placed in separate moist chambers for an equal length of time. One hundred spores from each specimen were measured, and again no significant differences in spore size, shape, or number of septa were found to exist between those samples taken from Pr and those taken from other inbreds.

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Pure cultures secured from each of the 16 diseased specimens were placed on individual lots of Pr seedlings in the greenhouse. The Pr inbred is susceptible to both races under greenhouse conditions, but in the field Pr was never found to be parasitized by a race of the fungus that was also capable of parasitizing other inbreds. Special care was exercised during and after inoculation to prevent spread of spores between the different lots of seedlings.

After one week the isolates from Pr had produced the characteristic symptoms constantly observed when this inbred is infected with that race of the species of *Helminthosporium* originally isolated from it. The isolates from other inbreds produced symptoms indistinguishable from each other but strikingly different from those produced on this inbred by isolates from Pr.

The individual lots of infected seedlings were harvested separately, dried, and placed in moist chambers. One hundred spores from each seedling lot were measured. The data obtained are presented in table 1. Based upon the analysis of variance, no significant differences in size were found between the two groups of cultures.

TAXONOMIC RELATIONSHIPS

Of the species of the genus *Helminthosporium* known to attack maize, *Helminthosporium maydis* Nisik. and Mke. appears to be, with certain minor exceptions, similar to the species under consideration. In 1925, Drechsler (2) described the ascigerous stage of a species of *Helminthosporium* under the name *Ophiobolus heterostrophus* Drechs. The name was later changed to *Cochliobolus heterostrophus* Drechs. (3). In 1926 Nisikado and Miyake (4) named the imperfect form of the fungus, *H. maydis.* Recently, Böning (1) reported the occurrence of *H. maydis* in Germany. Certain morphological characters of the species, as observed by Drechsler, Nisikado and Miyake, and Böning, are compared in table 2, together with the writer's findings for material examined from the Corn Belt.

Drechsler (2) states that the spores of the conidial stage of *Cochliobolus heterostrophus* exhibit rather pronounced curvature as compared with spores of other species of the genus *Helminthosporium.* Böning, however, found this untrue in the material he observed.

The symptoms as described and figured by Drechsler (2) are similar to those observed on inbred Pr under field conditions. The symptoms reported and illustrated by Böning, as occurring in Germany, resemble those found on inbreds other than Pr. Drechsler and Nisikado and Miyake were apparently able to infect almost any variety of corn without difficulty, suggesting that the isolates with which they worked showed no host specialization.

TABLE 1.—*Length, width, and septation among 100 spores of Helminthosporium in pure cultures of each of 16 collections grown on seedlings of corn inbred Pr*

Culture	Original host	Source of collection	Spore length		Spore width		Septa	
			Range	Mean and standard deviation	Range	Mean and standard deviation	Range	Mean
Number			*Microns*	*Microns*	*Microns*	*Microns*	*Number*	*Number*
Originally collected on inbred Pr								
1	Pr	Illinois	28–92	64 ± 11.0	9–16	13 ± 2.0	3–11	8
2	"	"	42–100	64 ± 14.0	9–16	12 ± 2.0	5–11	7
3	"	"	44–90	66 ± 10.0	9–16	12 ± 1.0	5–13	8
4	"	Indiana	37–92	63 ± 13.0	11–16	13 ± 1.0	4–11	8
5	"	"	31–95	64 ± 13.0	9–16	13 ± 1.0	4–12	8
6	"	"	35–92	64 ± 11.0	10–14	12 ± 1.0	5–12	8
7	"	"	37–87	63 ± 11.0	10–14	12 ± 1.0	3–10	8
8	"	"	40–96	68 ± 11.0	10–14	13 ± 1.0	5–12	8
Grand means and their standard errors				64 ± 2.1		12 ± 0.6		
Originally collected on inbred hosts other than Pr								
9	187–2	Indiana	35–88	65 ± 9.0	10–18	13 ± 1.0	5–11	8
10	P.I.[a]	Illinois	35–92	64 ± 13.0	10–18	12 ± 1.0	4–11	8
11	"	Indiana	28–94	67 ± 9.0	11–14	12 ± 1.0	5–12	8
12	"	"	31–87	61 ± 13.0	9–18	13 ± 1.0	3–11	7
13	187–2	Illinois	30–96	62 ± 15.0	7–16	12 ± 1.0	3–11	8
14	P.I.	"	28–92	60 ± 12.0	10–14	12 ± 1.0	4–11	8
15	"	"	40–87	61 ± 10.0	9–16	12 ± 1.0	5–12	8
16	"	Ohio	31–87	67 ± 12.0	10–14	13 ± 1.0	5–10	8
Grand means and their standard errors				63 ± 2.8		12 ± 0.6		

[a] Proprietary inbred.

TABLE 2.—*Comparative measurements, septation, and hilum character of conidia of Helminthosporium maydis from different sources*

Observer	Origin of material	Range of spore length	Range of spore width	Range of septations	Character of hilum
		Microns	*Microns*	*Number*	
Drechsler	Philippine Islands and Florida	20–115	10–17	3–12	Not conspicuous
Nisikado and Miyake	Japan	31–140	10–22	15[a]	"
Böning	Germany	46–128	12–17	7–10	"
Ullstrup	Indiana, Illinois, and Ohio	28–100	7–18	3–12	"

[a] Maximum.

Because of the similarity of certain fundamental characteristics, the fungus herein reported is referred to *Helminthosporium maydis.* However, the differences between isolates from Pr and those from other inbreds, with respect to virulence, host specialization, and symptoms, seem to justify separation. Consequently, that race restricted in its parasitism to inbred Pr is designated as *H. maydis* race 1. The race parasitizing a wide range of inbreds and hybrids, including a number that are grown commercially in the Corn Belt, and producing symptoms distinct from those caused by race 1 on Pr, is designated as *H. maydis* race 2.

Helminthosporium maydis Nisik. and Mke. Race 1:

Conidia olivaceous-brown; 28–100 µ × 7–18 µ (mostly 60–70 µ × 11–14 µ), widest at the center, and tapering toward the rounded ends; slightly curved, rarely markedly so; 3–13 septations (mostly 5–10); hilum inconspicuous. Spores germinating by 2 polar germ tubes. Conidiophores arising singly or in small groups from the stomata; olivaceous-brown, 90–230 µ × 5–7 µ, bearing one to several conidia at points marked by geniculations.

Highly virulent on all aerial parts of inbred Pr causing characteristic zonate lesions that frequently coalesce. No other inbreds or hybrids have been found susceptible.

Helminthosporium maydis Nisik. and Mke. Race 2:

Morphologically indistinguishable from race 1. Found on a number of inbreds in the field and able to parasitize all inbreds and hybrids tested as seedlings in the greenhouse. Distinguished from race 1 on basis of symptoms, which are characterized by narrow, elongated, chocolate-brown lesions that are marked by general coalescence. Race 2 is further differentiated from race 1 by being considerably less virulent and less specialized toward its hosts.

Helminthosporium turcicum Passerini is, perhaps, the commonest species of the genus found on maize in the Corn Belt. It is distinguished readily from *H. maydis* on morphological and symptomological bases.

Lesions caused by *Helminthosporium turcicum* are characterized by relatively few, large, olivaceous, elliptical spots on the leaves (Fig. 1). The fungus may destroy an appreciable amount of leaf tissue, but, apparently, is not so virulent as either of the races of *H. maydis.* So far as can be determined there is but one published report (5) of the occurrence of *H. turcicum* on the ears of maize in the United States.

Morphologically, *Helminthosporium turcicum* is distinguished from *H. maydis* in that the conidia are considerably greater in both length and width. Furthermore, the spores of *H. turcicum* show fewer septations, and the hilum protrudes conspicuously. (Fig. 2.)

Helminthosporium zeicola Stout was described by Stout (6) in Illinois in 1930. The fungus was found on the lower nodes of corn stalks in September and October. While, morphologically, this species is not strikingly different from *H. maydis,* Stout felt that the absence of a perithecial stage and the presence of the fungus on the stalk, rather than on the leaves, were sufficient reasons for describing a new species. It was suggested by Stout, but not demonstrated, that *H. zeicola* probably was pathogenic. Since the collections were made in the fall on mature or perhaps dead corn stalks, some may doubt its parasitic habit. Absence of the perfect stage and the caulicolous habit appear not to be valid criteria for taxonomic separation. Furthermore, even though the fungus were pathogenic, it appears from a purely morphological standpoint to be synonymous with *H. maydis,* and, on the basis of priority, should have been designated as such.

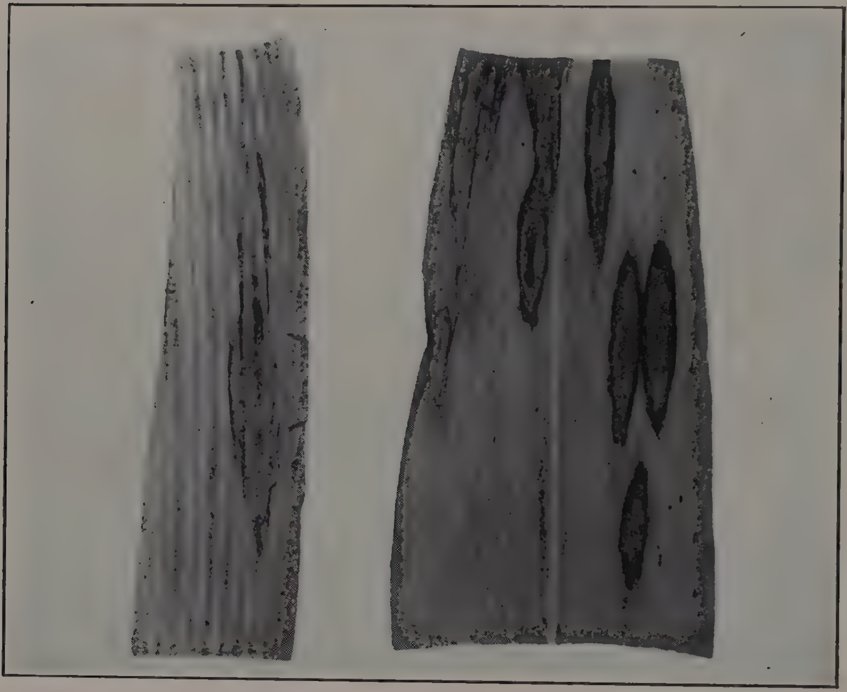

FIG. 1. Portions of fully developed leaves showing infection by *Helminthosporium turcicum.* × about ½.

Repeated searches for an ascigerous stage of *Helminthosporium maydis* have been conducted in both laboratory and field. Fresh and overwintered material, held for different periods of time and under different levels of moisture, have failed to give rise to an ascomycetous fungus that, from the standpoint of parasitism and morphology, could be designated as the ascigerous stage of *H. maydis.* Field observations on overwintered ears and leaves have likewise failed to reveal the perfect stage of this fungus.

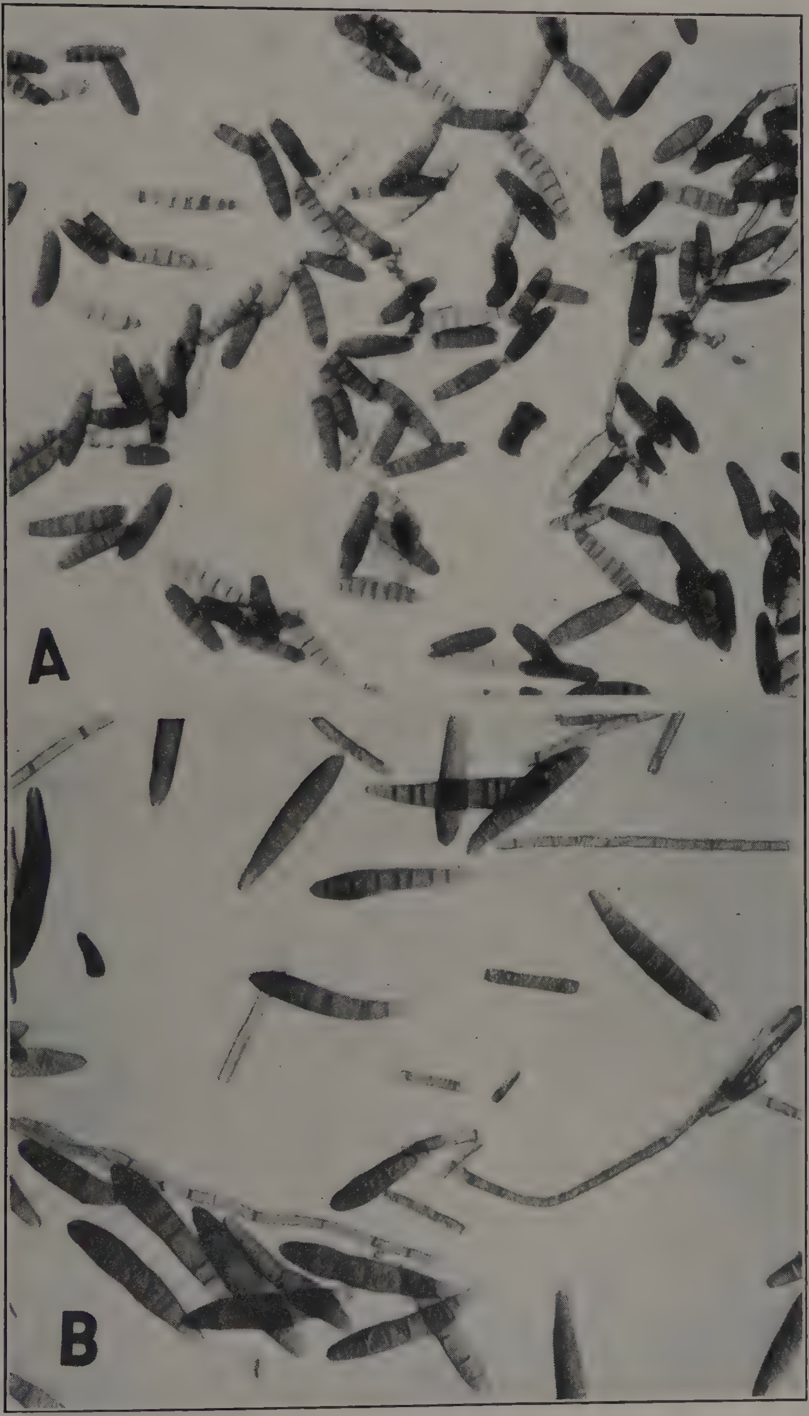

FIG. 2. A. Conidia of *Helminthosporium maydis*. B. Conidia of *H. turcicum*. × about 275.

SYMPTOMOLOGY

The symptoms on plants of inbred Pr, attacked by *Helminthosporium maydis* race 1, appear, in their early stages, as small, pale-green or yellowish spots on aerial portions of the plant. With the rapid enlargement of the spots the central portion dies and becomes straw-colored, with a dry, papery texture. The margins of the lesions show definite concentric zones varying from light- to purplish-brown. The lateral development of the parasite appears to be impeded somewhat by the parallel leaf veins, thus giving an oblong pattern to the lesions. (Fig. 3). On fully developed leaves in the field single lesions range in size from those less than 0.5 mm. in diameter and hardly visible, to those 5 × 20 mm. Under conditions of high humidity the fungus fruits abundantly on the surface of the host, giving a greenish-black cast to the infected areas. Sporulation is especially prolific on the leaf sheaths, husks, and about the stalk nodes. Ear infection (Fig. 4) results in the development of black mycelium over the kernels. Invasion may take place through either the tip or the butt of the ear or directly through the husk.

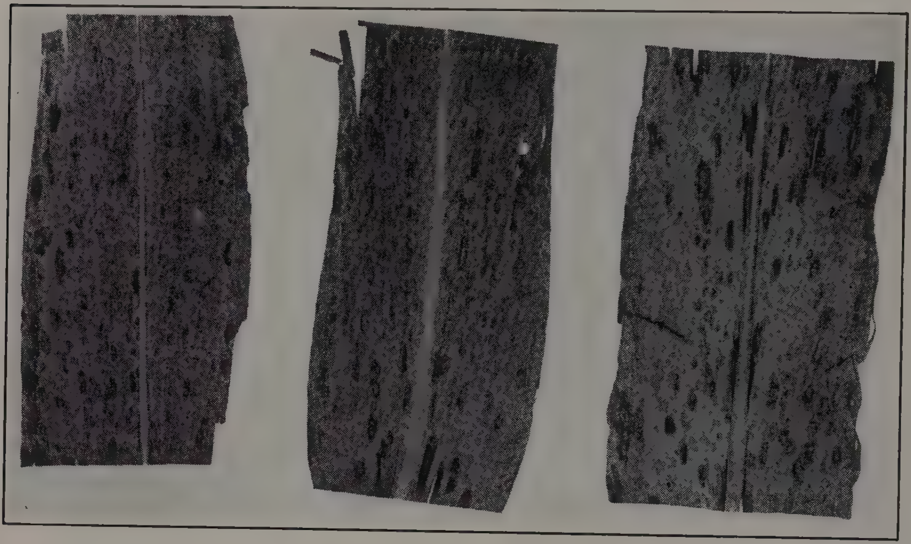

FIG. 3. Symptoms caused by *Helminthosporium maydis* race 1 on full grown leaves of Pr. × about $\frac{1}{2}$.

Infected plants, *en masse,* have a mottled, dead, grayish-green appearance as contrasted with the brilliant-green of resistant plants. This contrast is especially noticeable in crossing-fields where other inbreds are growing adjacent to the susceptible Pr.

The symptoms on greenhouse seedlings, artificially inoculated, are not strikingly different from those on field-grown plants. Very small, light-green areas may be observed on seedling leaves as early as 14 to 18 hours after a spore suspension has been atomized on the plants. The lesions enlarge rapidly with concomitant water-soaking in the central area. Within 7 days after inoculation the lesions are similar in appearance to those found on older plants in the field during the height of disease development (Fig. 5). The lateral spread of the fungus in seedling leaves does not appear to be checked by fibrovascular bundles to the extent observed on older plants, and the lesions are thus circular in outline.

The symptoms caused by race 1 on resistant inbreds and hybrids (Fig. 5, left) appear as small, yellowish-green flecks from 0.5 to 1 mm. in diameter. Such flecks have never enlarged and, in fact, have appeared to diminish in size as the leaves expanded.

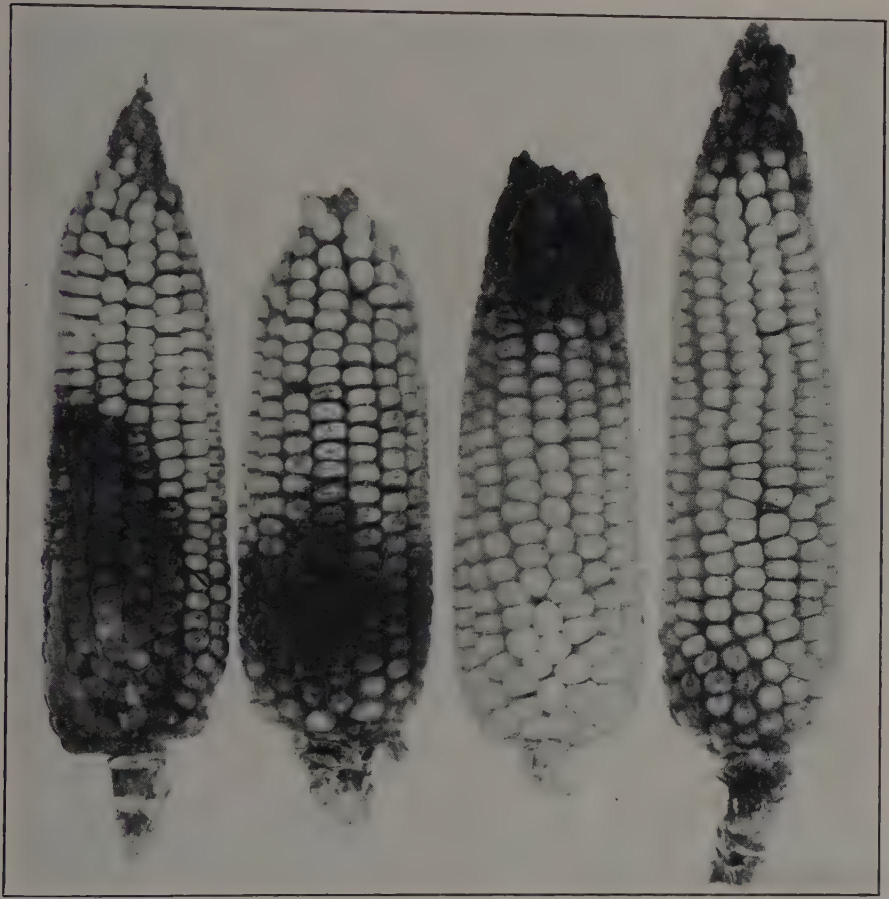

FIG. 4. Ear rot of inbred Pr caused by *Helminthosporium maydis* race 1. Ear rot of other inbreds caused by *H. maydis* race 2 is indistinguishable.

Examination of prepared sections of seedling leaves of Pr, fixed at different periods following inoculation with race 1 of the fungus, show penetration to take place either directly or through stomata, within 16 hours after spores are placed in contact with the leaves. The progress of the parasite in susceptible leaves is rapid; within 48 hours after inoculation relatively extensive areas of host cells are completely ramified by the fungus mycelium.

Penetration of resistant inbreds appears to be effected with equal facility. Subsequent progress of the parasite, however, is arrested soon after penetration and the fungus hyphae are confined chiefly to the epidermal cells.

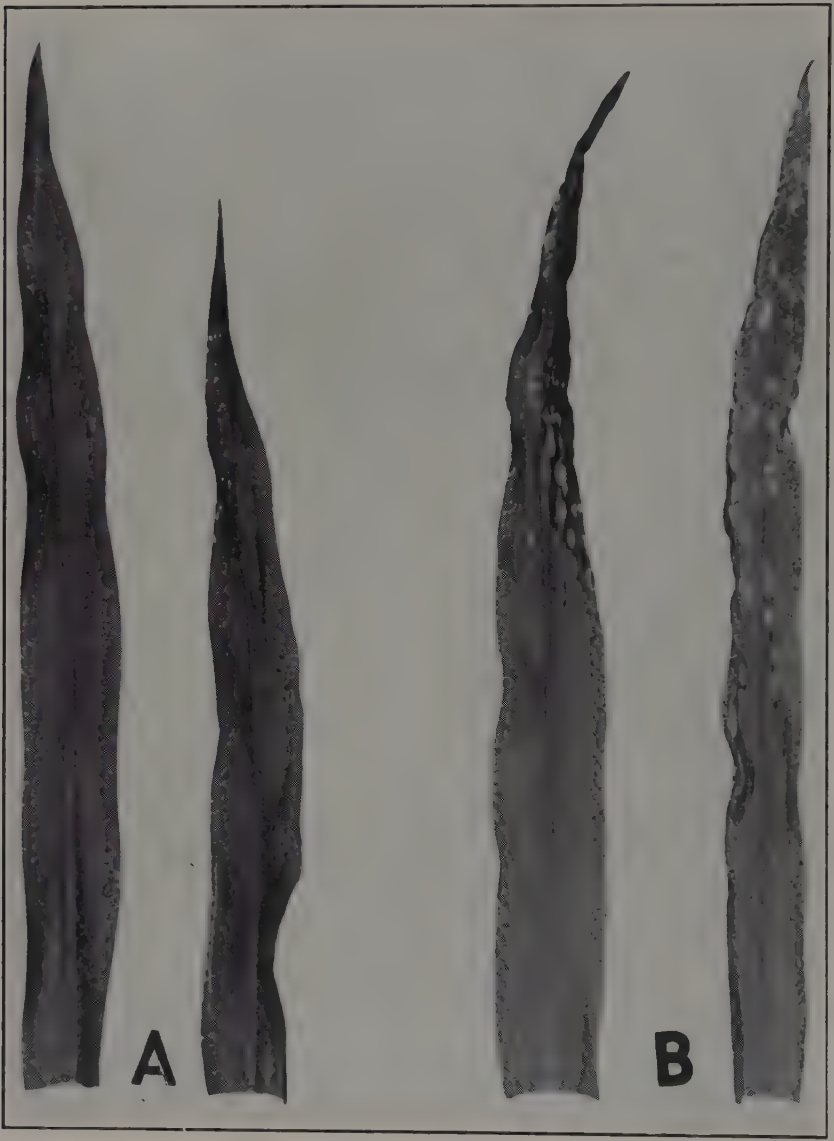

FIG. 5. A. Seedling leaves of a resistant inbred inoculated with *Helminthosporium maydis* race 1. Note small flecks where penetration has been effected but development of the parasite arrested. B. Seedling leaves of inbred Pr inoculated with *H. maydis* race 1. × about $\frac{2}{3}$.

Occasionally, however, a cell or two of the mesophyll have been found penetrated. Observation of sections of inoculated resistant leaves have failed to disclose any morphological barrier to account for the cessation of development of the parasite. It is suggested that resistance in this instance is of a physiological nature.

The symptoms produced by *Helminthosporium maydis* race 2 were first observed in 1939 on a proprietary inbred (Fig. 6), but were so unlike those found on inbred Pr that the disease was not suspected of being caused by a species of *Helminthosporium*. After plating diseased tissue and observing scrapings from infected areas, the disease was, however, recognized as traceable to a species of this genus. Reproduction of symptoms with pure culture inoculations established the fungus as the responsible parasite.

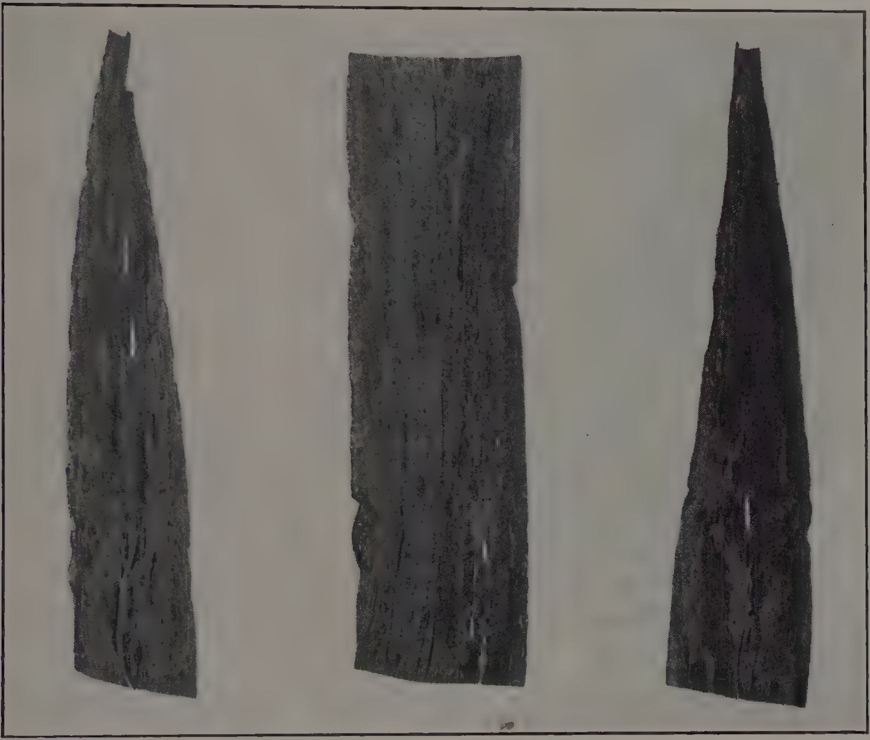

FIG. 6. Portions of fully developed leaves of a proprietary inbred infected by *Helminthosporium maydis* race 2. × about ½.

The symptoms in the field were characterized by irregular, elongated, chocolate-brown spots that coalesced to form extensive areas of dead tissue. Individual lesions on full-grown leaves ranged from mere flecks up to 3 × 20 mm. in size. Coalescence was especially marked toward the tips of the leaves.

Symptoms on artificially inoculated seedlings in the greenhouse are, at first, not easily distinguished from those on Pr seedlings caused by *Hel-*

minthosporium maydis race 1. Within 3 or 4 days, however, the general appearance of the infected areas change; and within 7 days narrow, elongated, dark-brown lesions without distinct zonation are in evidence. Symptoms on seedlings of inbred Pr inoculated with race 2 are no different from those found on other inbred seedlings when parasitized by this race.

Ears may be infected by race 2, as has been described for *Helminthosporium maydis* race 1 on Pr. The ear symptoms are indistinguishable from those caused by race 1.

INOCULATION STUDIES

The race classification of the fungus isolated from each specimen of diseased material collected was determined.

A spore suspension of a pure culture obtained from a given collection was atomized on seedlings of various inbreds and hybrids. The symptoms produced and the inbreds attacked served as a basis for classifying the isolates into either race 1 or race 2.

All isolates from inbred Pr parasitized the seedlings of this inbred with the production of characteristic symptoms as described above. All other inbreds and hybrids tested, including single crosses involving Pr, were highly resistant. These isolates were classified as race 1.

Isolates from other inbreds produced the symptoms described above for race 2 on seedlings of all inbreds and hybrids tested. Some differences in susceptibility to race 2 were apparent among the various lines of corn

TABLE 3.—*Reaction of certain inbreds and hybrids in the seedling stage to Helminthosporium maydis, race 1 and race 2*

Inbred of hybrid tested	Reaction of inbred or hybrid to—	
	Race 1	Race 2
Pr	Susceptible	Moderately susceptible
187–2[a, b]	Highly resistant	Susceptible
PI[b]	" "	"
P.H.[c]	" "	"
38–11	" "	Resistant
Ldg	" "	"
187–2	" "	Susceptible
4–8	" "	Resistant
66	" "	Moderately susceptible
L317	" "	Resistant
Hy	" "	Moderately resistant
A	" "	....
540	" "	....
R4	" "	....
90	" "	....
WF9	" "	....
M14	" "	....
Tr	" "	....
L	" "	....
Pr × Hy	" "	....
Pr × 38–11	" "	....
Pr × Tr	" "	....

[a] Recovered line of 187–2. [b] Proprietary inbred. [c] Proprietary hybrid.

inoculated, but none showed the high resistance to this race of the parasite that is characteristic of all inbreds and hybrids, with the exception of Pr, to race 1.

A list of the inbreds and hybrids of maize tested for susceptibility to the two races is given in table 3.

In addition to testing the virulence of the two races of the fungus on inbreds and hybrids of maize, a number of other hosts also were inoculated. These included wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), sugar cane (*Saccharum officinarum* L.), sorghum (*Sorghum vulgare* Pers.), Sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitch.), and rice (*Oryza sativa* L.). The small grains showed no evidence of infection by either race of the fungus, although susceptible inbreds of corn, inoculated at the same time and held under identical conditions, showed symptoms typical for each race. Cane, rice, sorghum, and Sudan grass showed minute purplish spots, which bore striking similarity to those produced on rice and cane when inoculated with *Ophiobolus heterostrophus* Drechs. (2). The two races could not be separated on the basis of symptoms produced on these hosts. Furthermore, purplish spots appear on sorghum and Sudan grass following any injury to the tissues.

Since the two races of the fungus were first observed, search has been made for wild-grass hosts of these parasites. Although a number of the grasses collected have shown infection by species of *Helminthosporium,* none of these parasites has proved parasitic on corn or has shown morphological similarity to *H. maydis.*

DISCUSSION

The apparently sudden and geographically widespread occurrence of *Helminthosporium maydis* race 1 in 1938 is difficult to explain. No report can be found in the literature of a specialized race of this species of the fungus, and it is doubtful whether any important outbreak of the disease caused by this race occurred prior to 1938. The abundance of moisture during the early spring and summer of that year undoubtedly played an important role in the spread and development of the disease.

Seedlings arising from diseased kernels show abundant fruiting of the fungus near the soil line under conditions of high humidity. This has been observed, even when the seed has been treated with an organic mercury dust. Kernels that show no macroscopic evidence of infection frequently yield the fungus when surface-sterilized and plated on agar substrates. The sudden and widespread occurrence of the disease may thus possibly be explained by the fact that the fungus is carried on or within the seed. The establishment of the disease in any given area, however, is contingent upon proper environmental conditions.

Although any explanation of the origin of this highly specialized race would be lacking in proof at the present time, certain hypotheses may be mentioned. If this race is indigenous to the Corn Belt it may have existed as a parasite on open-pollinated corn; but, because of the heterozygosity of such corn, few plants may have been attacked and, as a consequence, escaped notice. Another possibility is that this race existed as a saprophyte and became parasitic only when an inbred, homozygous for susceptibility, was produced. It is also conceivable that this race may be a mutant type that arose from the less specialized form that has been reported from Florida (2).

It is possible that race 2 may be indigenous to the Corn Belt, but escaped notice because of its low degree of virulence. Its presence becomes conspicuous under circumstances of abundant moisture together with a relatively susceptible host.[3] This race of the fungus also appears to be disseminated by carriage on or within the seed.

SUMMARY

A species of *Helminthosporium* was observed on certain inbred lines of maize in Indiana, Illinois, and Ohio in 1938 and 1939, which appears to be morphologically identical with *H. maydis* Nisik. and Mke.

Two morphologically indistinguishable races of the species were recognized on the basis of host specialization, symptoms produced, and virulence.

It is proposed that race 1 of *Helminthosporium maydis* include the parasite that has thus far been limited in its parasitism to only the one inbred, Pr, and that produces the distinctive symptoms on this inbred, as described.

All isolates from Pr attacked only this inbred; other inbreds and hybrids tested, including single crosses involving Pr, being highly resistant.

It is proposed that race 2 of *Helminthosporium maydis* include the parasite of a somewhat wider host range, which, under field conditions, has been observed on inbred 187–2 and a number of proprietary inbreds, and produces the characteristic symptoms, as described, distinct from those produced by race 2 on inbred Pr. Race 2 has been found to be less virulent on its inbred hosts than race 1 on Pr.

Hyphae of both races of the fungus penetrated the leaves of sugar cane, rice, sorghum, and Sudan grass and this was followed by the development of small purplish spots. The races are not separable on the basis of symptoms found on these hosts.

It is suggested that race 1 may be indigenous to the Corn Belt and has been parasitic on open-pollinated corn, but escaped notice because only a few plants of such heterozygous corn were attacked. The fungus also may have existed largely as a saprophyte until an inbred, homozygous for susceptibility, was produced. Another possibility of the origin of race 1 suggested is that it arose as a mutant from the less specialized type known to occur in Florida.

Race 2 also may be indigenous to the Corn Belt, but, because of low virulence, escaped recognition until proper environmental conditions ensued and a relatively susceptible host was present.

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[3] Through the courtesy of J. G. Dickson and E. Stevenson the writer had opportunity to examine a pure culture of *Helminthosporium maydis* isolated by them from material collected in southwestern Wisconsin. On the basis of symptoms it produced and inbreds attacked, this culture could be classed as *H. maydis*, race 2.

Both races appear to be spread by means of infected seed.

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LITERATURE CITED

1. BÖNING, K. Helminthosporiosen an Mais. Prakt. Bl. Pflanzenb. **16**: 159–167. 1938.
2. DRECHSLER, CHARLES. Leafspot of maize caused by *Ophiobolus heterostrophus* n. sp., the ascigerous stage of a *Helminthosporium* exhibiting bipolar germination. Jour. Agr. Res. [U. S.] **31**: 701–726. 1925.
3. ————. Phytopathological and taxonomic aspects of *Ophiobolus, Pyrenophora, Helminthosporium,* and a new genus, *Cochliobolus.* Phytopath. **24**: 953–983. 1934.
4. NISIKADO, Y., and C. MIYAKE. Studies on two diseases caused by *Helminthosporium turcicum* Passerini and *Ophiobolus heterostrophus* Drechs. = *H. maydis* Nisikado et Miyake. Berichte Ohara Inst. für landwirtsch. Forsch. **2**: 221–266. 1926.
5. SHERBAKOFF, C. D., and L. S. MAYER. Black ear rot of corn. Phytopath. **27**: 207. 1937.
6. STOUT, G. L. New fungi found on the Indian corn plant in Illinois. Mycologia **22**: 271–287. 1930.

EXPERIMENTAL PRODUCTION OF SYMPTOMS IN SO-CALLED RECOVERED RING-SPOT TOBACCO PLANTS AND ITS BEARING ON ACQUIRED IMMUNITY[1, 2]

W. D. Valleau

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Since Wingard's (12) observations that tobacco plants with the ring-spot virus disease gradually take on more normal growth without ring patterns and "seem to develop an immunity to the ring-spot disease," Price (4, 5, 6, 7), in a series of papers, has supported the claim that such plants actually recover and, because second inoculation does not repeat the symptoms of the first, they acquire immunity. The assumption that plants acquire immunity from viruses as a result of a previous attack, and that "the principles involved are the same as those made use of in human and animal pathology for immunization by vaccination" (3), is one that plant pathologists should study with great care before accepting. At the present time there are those who accept this assumption as a proved fact, while there are others, equally familiar with the facts, who see in the evidence presented nothing fundamentally different from what has been known of fungus and bacterial plant diseases for years.

The acquired-immunity assumption is about as follows: A group of cells of a plant invaded by a virus is considered immune from the virus already present, or from a closely related strain of the same virus, because second inoculation does not repeat in the invaded tissue the symptoms characteristic of those usually produced by primary infection with the second virus.

Would one expect to be able to produce a second perfect stand of bluegrass by reseeding a lawn that already has a perfect stand of grass? If this could not be done, would the soil be considered to have acquired an immunity from bluegrass? In the writer's opinion the two phenomena are comparable.

This paper is concerned with the symptoms produced by tobacco plants that have passed the typical ring-spot stage, and the bearing of these symptoms on the assumption that the plants have recovered and have acquired immunity from the virus.

VIRUS STRAINS

During the winter of 1939–40, a study was made of 6 strains of the ring-spot virus. Four were collected in the field in 1939 by saving seed from ring-spot affected plants, 1 was received from S. G. Lehman, North Carolina, and another was the yellow ring-spot virus, previously studied by the writer (8), which had been stored in seed. Four strains caused chlorosis of seedlings, when seed-transmitted, and varying degrees of yellowing of leaves of

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] King award paper, Kentucky Academy of Science, 1940.

so-called "recovered" plants; one caused very slight bleaching of old leaves, and another was non-yellowing. Each strain produced slightly different symptoms from each of the others and may be considered to be distinct (Table 1, Fig. 1, A–D). The fact that in random collection of ring spot 6 distinct strains were found, no two of which affected plants similarly, illustrates the complexity of the strain problem and the present impossibility of describing strains with sufficient accuracy to enable one to distinguish newly collected strains from those already described. Confusion would arise if each new strain were given a Latinized varietal name, as Holmes has proposed.

All 6 strains were transmitted through seed and transferred from affected seedlings to Kentucky No. 16 Burley plants. These were then set in duplicate in a ground bench in the greenhouse. Opportunity was taken during this study to vary the temperature in the house in order to produce, if possible, the leaf-edge symptoms on so-called recovered plants and to note the effect, if they developed, of the different strains of virus on the severity of symptoms.

TABLE 1.—*Comparison of six strains of ring-spot virus on No. 16 Burley tobacco*

Virus strain	Seed transmission	Color of seedlings	Field symptoms	Symptoms of inoculated plants	Injury in "recovered" leaves at reduced temperature
92–1	+[a]	Yellow	Yellow patterns	Patterns necrotic to chlorotic	++[b]
92–9	+	Yellow	Necrotic patterns	Severe necrosis and distortion	+
92–10	+	Yellow	"Recovered" portion yellow	Slight necrosis and yellow patterns, no distortion	–[e]
92–11	+	Yellow	"Recovered" portion yellow	Severe necrosis and distortion	+
92–4	+	Green	Patterns green to necrotic	Patterns necrotic	+++[c]
92–7	+	Green	Patterns green to necrotic, nearly completely sterile	Severe necrosis and distortion, "recovered" leaves bleached slightly	++++[d]

[a] + Virus transmitted through seed.
[b] ++ Indicates mild leaf-edge chlorosis and necrosis.
[c] +++ Necrosis.
[d] ++++ Indicates very severe injury.
[e] – Indicates slight necrosis and yellow patterns; no distortion.

PRODUCTION OF LEAF-EDGE CHLOROSIS AND NECROSIS IN SO-CALLED RECOVERED PLANTS

The plants were inoculated and set in the ground bench January 3, 1940. Severe necrotic distorting ring spot developed in 5 pairs of plants, causing stunting. The 2 plants inoculated with the original yellow ring-spot virus

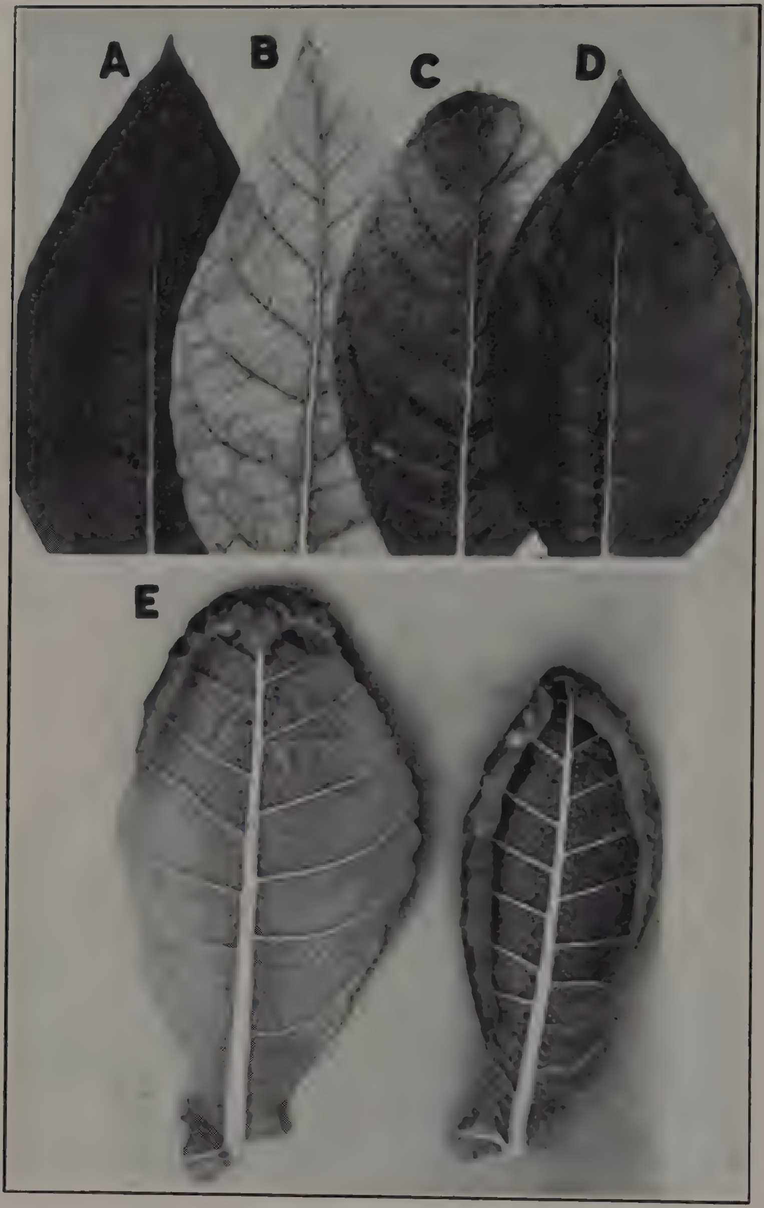

FIG. 1. A–D. Effect of 3 yellowing strains of ring-spot virus on comparable leaves of Kentucky No. 16 Burley tobacco plants in the so-called recovered stage in comparison with a leaf A from a healthy plant. Leaf B is affected with the original yellow ring spot strain, leaves C and D, with strains collected in 1939 in seed. The differences in chlorosis shown in leaves C and D are typical for the other leaves of these plants. The fourth yellowing strain studied caused chlorosis intermediate between leaves B and C. Note the pinched tip on leaf C caused by reduced temperature when the leaf was small. E. Leaves from plants affected with a non-yellowing strain of ring-spot virus. The tip and edge symptoms developed at a temperature of about 20° C. after which the temperature was raised to about 25° C., when rapid expansion of the leaves occurred, except in the affected border tissue. Photographed March 4, 1940.

developed yellow patterns with very slight necrosis and no stunting (92–10, Table 1). These 2 plants grew as rapidly as did the check pair throughout the test, and showed no further leaf symptoms other than general chlorosis of the leaves soon after formation (Fig. 2, A). By January 27, all plants were making growth free from ring-spot patterns and had entered the stage of so-called recovery. The temperature of the greenhouse during this time had been maintained at about 20° C., measured just above ground level in the shade of a leaf. The new leaves of 5 pairs of affected plants all developed tip and leaf-edge chlorosis and necrosis, the severity of which was in direct proportion to the degree of necrosis during the ring-spot stage. One yellowing strain (92–11, Table 1) caused slight leaf-edge symptoms and less bleaching of older leaves than the original yellow strain. Two other yellowing strains caused prominent leaf-edge chlorosis and necrosis and still less bleaching of old leaves. The slight yellowing (92–7, Table 1) and the non-yellowing strain (92–4, Table 1) caused severe leaf-edge symptoms of nearly the same degree (Fig. 2, B).

On February 16 the temperature was raised, varying from 24 to 28° C., just above the surface of the ground. During 9 days' exposure to this temperature leaves with tip and edge symptoms increased in size rapidly, but the more severely affected leaves hooked back sharply at the tips and edges, making a cup of the under side of each leaf (Fig. 1, B). All new leaves were apparently normal.

On February 25 the temperature was again reduced to about 20° C. for the ensuing 4 days. In 2 days the growing-point leaves of the plants that had not reached the blooming stage again showed leaf-edge necrosis or chlorosis, and leaves about $\frac{1}{4}$ grown developed prominent leaf-edge chlorosis. The degree of necrosis and chlorosis corresponded to that which developed during the earlier low temperature period. On February 29 the temperature was again raised to about 25° C., and subsequent new growth was normal. During the entire period the 2 noninoculated plants and all other plants in the greenhouse made normal growth.[3]

During the second period of low temperature, potted seedlings that had been infected with a non-yellowing strain of virus through the seed, but were on a bench with bottom heat and, consequently, making about normal growth, were set on the ground bench and subjected to the low temperature. In 3 days they had developed distinct chlorosis of the tip thirds of the younger leaves and would be readily recognized as infected with the ring-spot virus, although they had never produced ring patterns.

[3] From July 1 to 18, 1940, the average minimum temperature at Lexington was 13° C. During and following this period several "recovered" ring-spot plants were seen in the field that showed typical cold effect in the young leaves. The blade tissue of the basal half of one and $\frac{4}{5}$ of another leaf were necrotic. The tip portion of each of these leaves bore typical ring spot patterns, while the remaining necrotic portions were in the "recovered" zone of the plant. The blade of a third was completely necrotic, while the tips of 3 smaller leaves were necrotic. The "recovered" portion no longer developed ring-spot patterns, but was so severely injured as to be valueless.

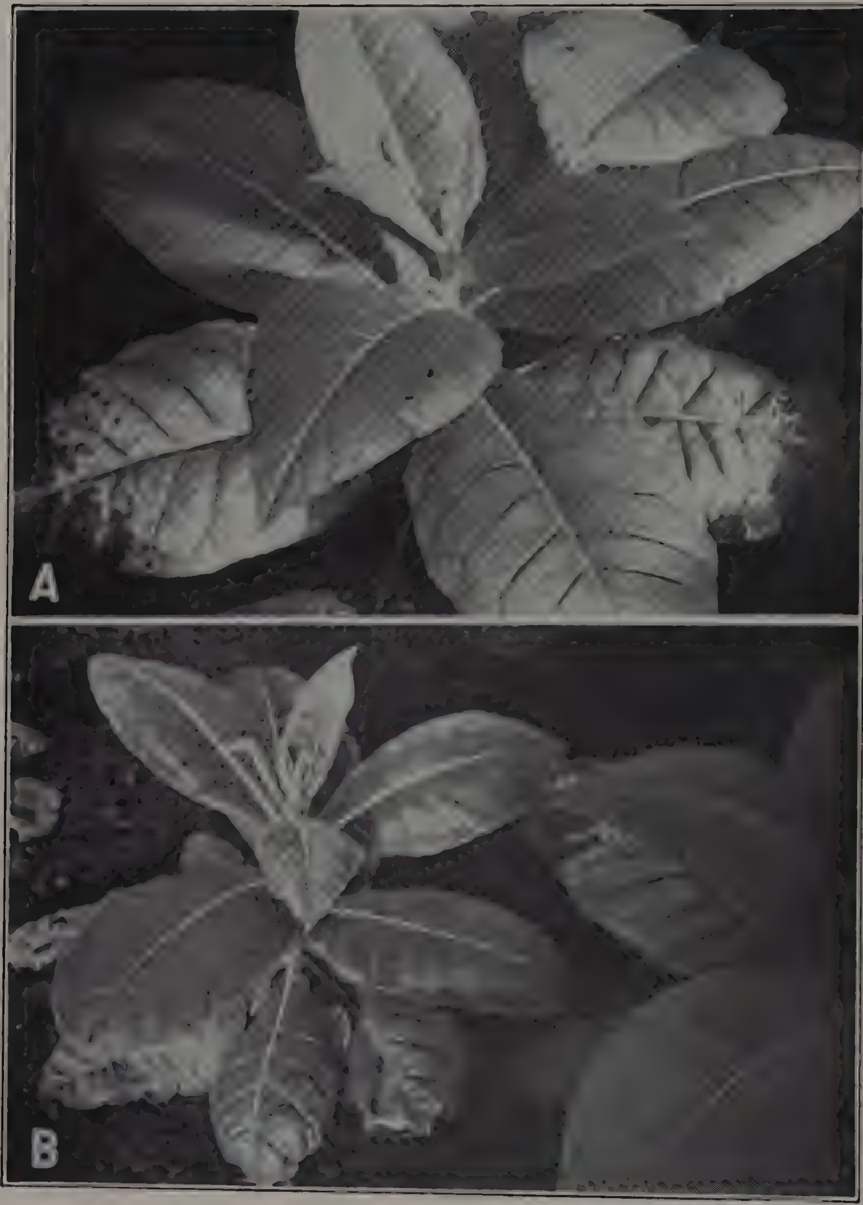

FIG. 2. A. Plant of No. 16 Burley affected with original strain yellow ring spot and grown at a temperature of about 20° C. The tips of the leaves at the left and right are of the normal green color; the remainder of these leaves and those above are all chlorotic. The low temperature caused no leaf edge symptoms on plants affected with this strain of the virus. Print from Kodachrome on direct positive paper. B. Plant 192–71 of Kentucky No. 16 Burley tobacco inoculated when set in the ground bench January 3, with a faintly yellowing strain of ring spot. The temperature was maintained at about 20° C. Two necrotic distorted ring spotted leaves can be seen at the left. The middle "recovered" leaves are curled downward along the edges and are chlorotic between the secondary veins. The upper leaves are chlorotic to necrotic and distorted. At the right is a plant affected with the original strain of yellow ring spot. The leaves of the latter have spread normally and, except for the gradual bleaching, would be considered normal.

POLLEN STERILITY

Following blooming, the anthers and pollen of healthy and affected plants were examined. Healthy plants produced abundant pollen, which resulted in a normal set of seed. The yellow ring-spot plants (92–10) produced a moderate but reduced amount of pollen, which proved to be over 9 per cent aborted. Each of the remaining 5 strains of the virus caused a high degree of pollen sterility, varying from apparent complete absence of pollen in numerous flowers examined to an occasional flower in which an appreciable amount of pollen was present. The filaments on affected plants were frequently too short to reach the stigma, whereas normal filaments place the anthers on a level with the stigma.

SEED SET

The effect of pollen sterility on set of seed is shown in table 2 and figure 3. The seed heads from ring-spot plants were in most instances larger than those on healthy plants because of continued growth and blooming, which followed failure of seed set, so that differences in seed set are actually greater than are shown. Because of poor pollen development, numerous blossoms failed to set and fell off; or, if they set, the pods contained few seeds and the ovary walls were shrunken and wrinkled. Certain strains of the virus caused almost complete sterility; others resulted in from few to a normal set of pods, but with a greatly reduced number of seeds per pod. The original strain of yellow ring spot, which caused no distortion of leaves at reduced temperature, following so-called recovery, set the largest amount of seed of the affected plants (92–10, Table 2).

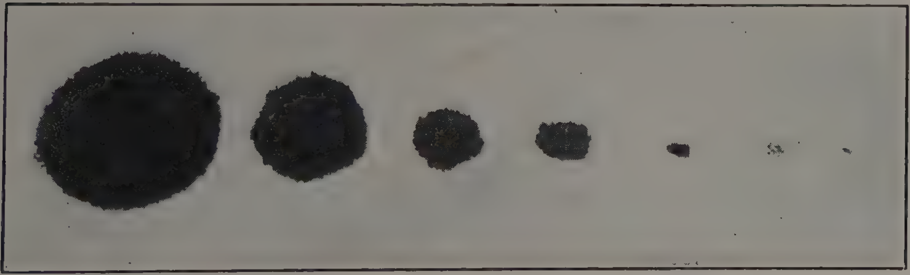

FIG. 3. Yield of seed from healthy and ring-spot tobacco plants. Left to right: Healthy, 92–10, 92–1, 92–9, 92–11, 92–4 and 92–7.

DISCUSSION

In defending the assumption that ring-spot-affected plants recover from the disease and acquire immunity, Price, in his recent paper (7), completely ignored evidence pertinent to the question. This evidence is as follows: The yellowing strains of the virus, when seed-transmitted, produce in very young seedlings striking chlorosis that persists to a greater or less degree throughout life, and produces a condition that anyone would recognize as disease,

TABLE 2.—*Yield of seed from a healthy plant and from plants infected with different strains of the ring-spot virus*

Plant	Pods	Total seed	Average seed per pod	Comments
	No.	*grams*	*grams*	
Healthy	86	10.0	0.116	....
92–10, pl. 1	17	0.4	0.023	Primary flower stalk
92–10, pl. 1	62	2.2	0.035	Lateral flower stalk
92–10, pl. 2	23	1.0	0.043	....
92–9, pl. 1	14	0.16	0.011	....
92–9, pl. 4	60	1.75	0.029	....
92–4, pl. 1	1	....	....	31 seeds, primary flower stalk
92–4, pl. 1	12	0.08	0.007	Lateral flower stalk
92–4, pl. 2	80	0.47	0.06	....
92–1, pl. 2	78	1.26	0.016	....
92–11, pl. 1	0	0.0	0.0	....
92–11, pl. 2	1	....	....	116 seeds
92–7, pl. 1	Few	....	....	3 to 10 seeds per pod
92–7, pl. 2	0	0.0	0.0	Primary flower stalk
92–7, pl. 2	1	0.0	0.0	Secondary flower stalk

even in the entire absence of any ring patterns. The non-yellowing strains and some yellowing strains produce easily recognizable symptoms in infected seedlings subjected to a temperature of about 20° C. These symptoms can be reproduced at any time during the growth of the plant by subjecting it to reduced temperature. The relation of low temperature was pointed out by Valleau in 1932 (8). At that time Price also recognized leaf-edge necrosis and chlorosis as a symptom sometimes occurring on the leaves of "recovered" plants (4). Pollen sterility is caused by all strains thus far studied. This symptom can be seen by examining anthers, which frequently contain no pollen; by examining for aborted pollen grains where there appears to be an abundant supply; or by examining seed pods, which are frequently shrunken or, if not shrunken, contain an abnormally small number of seeds. Price (7) admits that "recovered plants appear to grow somewhat less rapidly than healthy plants" and have leaves that are "slightly darker in color, somewhat thicker and of a more leathery texture." But it would seem that the only symptom of the ring-spot disease which he is willing to recognize, in connection with the assumption of acquired immunity, is ring and line patterns.

Yet there is good reason to believe that ring-spot-affected plants can be kept continuously in an abnormal condition (Fig. 2, B) if grown at about 20° C. following so-called recovery. Leaf-edge chlorosis, necrosis, distortion, and pollen sterility are as definite symptoms of disease as ring patterns; plants, therefore, cannot be considered to have recovered when ring patterns are no longer produced. The conclusion seems inevitable that there are 2 distinct phases of the disease: 1. That in which mature, relatively old tissue is invaded, following inoculation, with the production of ring and line patterns, as the virus is carried to and scattered in leaves higher and higher on the plant. During this period only the patterned tissue or that included within a pattern is viruliferous. The remaining parts of affected leaves are virus-free and healthy. 2. That in which embryonic or at least very young tissue is invaded, resulting in nearly uniformly invaded leaves without ring patterns. These leaves become slowly or rapidly chlorotic, depending on the strain of virus (yellow) present (Fig. 1, A–D, and 2, A); or develop more or less severe leaf-edge symptoms, depending upon the virus strain, if the plants are grown at a temperature below optimum (Fig. 2, B); or take on the appearance of normal healthy leaves if the virus is non-yellowing and the plant is grown at a temperature of about 25° C. or above.

A sudden change in symptoms following invasion of young tissue is not limited to tobacco ring spot. Tomato plants affected with mixed virus streak develop severe streaking during the period of invasion of healthy tissue but, later, when very young leaves are being invaded, such plants develop mosaic mottle with little if any necrosis or streak. Tobacco plants, affected with a burning strain of tobacco-mosaic virus, develop severe necrosis in young leaves just too old to develop mottle patterns; but younger leaves develop typical mosaic mottle with little if any necrosis (11). Tobacco plants infected with the tobacco-streak virus develop severe necrosis of the stalk and younger leaves and slow necrosis of older leaves, as they are invaded, but, following invasion of embryonic tissue, no further necrosis occurs; a speckled type of mottling develops (2), hardly visible in plants growing in the field. Greenhouse-grown plants, however, are frequently prominently mottled and, as such, should not be considered recovered or healthy. Potato plants affected by the yellow-dwarf virus develop severe necrosis as the virus invades mature tissue, but, following invasion of embryonic tissue, take on a set of symptoms properly termed chronic by Black (1). While Price (7) lists yellow dwarf as one of the diseases from which plants "recover" it is worthy of note that potato growers are not willing to plant "seed" from so-called recovered plants in commercial plantings as a means of controlling the disease. It seems obvious that plant parts, infected when mature, are affected by the virus in a manner different from that affecting those plant parts developing from tissue infected in the embryonic stage. But are we justified in concluding that the plants recover simply because the second set of symptoms differ from the first?

Price (7) has divided acquired immunity into the chronic-disease type, and the carrier type. Tobacco mosaic is a good example of the chronic-disease type. The plant obviously does not recover from tobacco mosaic as the virus continues to lag somewhat behind the embryonic tissue of the growing point; therefore, the plant continues to produce patterns. In the carrier type, represented by ring spot, the virus finally invades at least a part of the embryonic tissue of the growing point, and the subsequent leaves are more or less uniformly invaded as they develop and produce no definite ring patterns. There is then no fundamental difference between the chronic-disease type and the carrier type, the difference being apparently in the extent to which embryonic tissue is invaded in the two diseases and the ability of the viruses to produce an obvious effect in young leaves.

Price (5, 7) has reasoned that, because the so-called recovered leaves of ring-spot plants contain only $\frac{1}{5}$ as much virus as the patterned leaves, this is added evidence that the plant has developed immunity. He states "The cells of recovered plants are not, as Valleau suggested, parasitized to the same limit as the cells of diseased plants" (5). This reference is to a criticism of the acquired immunity theory by Valleau (9). The statement referred to by Price is as follows: "Inoculation of patternless leaves is without effect, as the cells are already parasitized to the limit." This statement makes no comparison of virus content of ring-spotted tissue with "recovered" tissue, but merely refers to the fact that the addition of one more virus particle to a cell in which the virus has already multiplied to its *then possible limit* should have no visible effect. It has not been demonstrated that the difference in virus content between cells invaded when mature and those invaded in the embryonic stage, is proof of the development of immunity. Is there any reason to suppose that cells, parasitized at the time of, or very soon after formation, and, consequently, diseased throughout life, should be capable of producing virus-precursor materials in as great quantity as cells that were invaded by virus when in a mature, vigorous condition and well-stocked with virus precursor? The difference in virus content in so-called recovered leaves and in patterned leaves may be in direct proportion to the virus-precursor material present when the cells were first invaded. It is not beyond reason that hypersensitivity of mature tissue to certain viruses, including ring spot, is the direct result of sudden change of a considerable quantity of precursor material into virus, resulting in an upset within the cell sufficient to cause its death; whereas the cell readily adjusts itself to the gradual change that takes place if infected in the embryonic stage and precursor material be gradually changed to virus.

The whole discussion of whether or not a plant develops immunity from a virus necessarily hinges on the definition of immunity. Immunity as defined in the Encyclopedia Britannica, 14th Edition, is as follows: "The term immunity is used in science in the technical sense. An animal is described as naturally immune against the microbe of a disease if the microbe in question cannot establish itself in the organism, and is artificially immune if it was naturally susceptible and has been rendered insusceptible." If we accept this definition for immunity, it is only necessary to show that the newly formed tissue of a tobacco plant that has "recovered" from ring spot is constantly being invaded by the virus, to prove that the plant is still susceptible and, therefore, by definition, cannot be considered immune. Price (5) demonstrated by his series of 10 successive cuttings from ring-spot plants a fact that was already known, namely, that the virus continues to multiply indefinitely in "recovered" ring-spot plants as they make new growth. The newly formed cells soon become invaded and, therefore, must be considered susceptible.

Price (7) defines acquired immunity as "the specific *resistance* an individual organism acquires to a disease." This definition seems to mean that immunity is resistance to the development of symptoms. This is apparently a new use for both the terms immunity and resistance as both are commonly thought of in connection with the relation between the host and the parasite rather than with respect to the development of specific symptoms. Does the definition mean that immunity and resistance are synonymous? and is it the disease or the causal agent of the disease to which an organism (plant) acquires resistance or immunity? or are disease and causal agent considered synonymous? If resistance and immunity are considered synonymous should the definition for immunity or for resistance apply? If resistance to the causal agent is implied, what degree of resistance constitutes immunity? If resistance to the development of symptoms of the disease is meant, how much must symptoms be suppressed before the plant can be considered immune? What is meant by the word specific? Does it refer to specific resistance of the plant to the development of a given symptom, such as the ring-spot symptom, and disregard all other symptoms, such as general chlorosis and pollen sterility? It would seem that these questions must be cleared up before this definition of acquired immunity can have any meaning.

What is the actual evidence that ring-spot plants develop immunity from reinfection by other strains of the same virus? Affected leaves of a tobacco plant, in the so-called acute or ring-spot stage, are not immune from reinfection, for, if one will inoculate the normal blade tissues between necrotic spots or rings, as the writer has done on several occasions, with the same virus, or with another strain, the second inoculation will be effective and will cause a second set of spots to develop. The tissues between the spots are uninvaded by virus and, consequently, are as susceptible as similar tissue on a noninoculated plant. It may, therefore, be concluded that plants in the acute or ring-spot stage are not immune.

If the plant as a whole is to be considered to develop immunity following so-called recovery, then new growth originating from any part of the plant should be immune. Valleau (10) demonstrated that an inoculated tobacco plant may "recover," and, as much as a year later, if cut back to a dormant bud, may push out a virus-free shoot, which finally becomes invaded, develops typical ring patterns, and ultimately "recovers" again. He (8) also demonstrated that a plant infected through seed with ring-spot virus may grow for a year without producing any ring patterns and, then, when cut back to a dormant bud, produce a shoot in which typical ring patterns are produced, followed by so-called recovery. Therefore, it may be concluded that the plant as a whole is not immune from the virus already present but may develop virus-free susceptible tissue that is finally invaded in exactly the same way as a healthy, recently inoculated, plant.

That not even the "recovered" portion of a ring-spot plant is immune from reinfection by another strain of the ring-spot virus is brought out by studies reported by Price. He (7) demonstrated that cuttings of plants that had "recovered" from what he called ring spot No. 1 and green ring spot developed chlorosis slowly, after inoculation with the yellow ring-spot virus (same virus as 92–10, Table 1) not only at the points of inoculation but in leaves higher on the plant, until finally the plants showed chlorosis typical of yellow ring spot in all subsequent growth. Apparently, the yellow ring-spot virus had largely replaced the original virus. It is obvious that the plants had neither been rendered immune from yellow ring spot virus by "recovery" from ring spot No. 1 or green ring spot, nor had they been protected against infection by the second strain of the ring-spot virus. Yet, there seems to be no doubt that all 3 strains are closely related. The obvious explanation seems to be that the second virus was able to become established in an already invaded leaf and, because of superior qualities of reproduction, was able gradually to supplant the first virus. Price claimed that yellow ring spot gave complete protection against ring spot No. 1 and green ring spot and that the latter two gave complete protection against one another, and concluded that all three viruses were closely related. There is, however, no proof that the second virus (non-yellowing) in each case did not become established in yellow ring-spot plants, just as the yellow ring-spot virus had, but could not manifest itself because of inability to cause necrotic ring patterns in tissue already invaded by a closely similar strain, because it lacked the ability to cause chlorosis or because of inferior qualities of reproduction. The writer succeeded in producing yellow spots on "recovered" leaves of a Burley plant infected with ring-spot virus (92–4, Table 1) by inoculating with the yellow ring-spot virus. It may be tentatively concluded that "recovered" invaded tissue is not actually completely protected against reinfection by a second strain of the same virus. This conclusion naturally raises the question as to the value of the so-called immunity reaction in the classification of strains of the ring-spot virus in instances where complete protection is not afforded.

A critical analysis of other reported cases of the development of immunity in plants by infection with one strain of a virus against other strains will lead to the conclusion that the phenomena can be more easily and satisfactorily explained by competition between virus strains than by assuming the development of immune bodies, such as seem to develop in animals rendered immune from reinfection by a previous attack, even though the location of the immune bodies be considered limited entirely to invaded tissue.

CONCLUSIONS

Six strains of the ring-spot virus collected at random proved to be distinct from one another, suggesting that numerous strains exist in nature. The inadvisability of applying a Latinized trinomial to each strain is mentioned.

Tobacco plants that have "recovered" from the ring-spot stage produced by 5 strains of the virus developed prominent leaf chlorosis and necrosis when subjected to a temperature of about 20° C., whereas nearly normal growth occurred at 26° C. This appears to be proof that the plants do not recover from ring spot, but simply enter a different phase of the disease.

Each of the 6 strains of virus caused pollen sterility, which greatly reduced the quantity of seed produced. If seed were the chief commercial product of the tobacco plant sterile plants would not be recognized as having recovered.

Tobacco plants in the acute ring-spot stage are not immune from reinfection in uninvaded tissue. Plants that have "recovered" may produce shoots that finally develop typical ring spot, indicating that the plant as a whole is not immune following "recovery." Plants that have "recovered" are still apparently susceptible to reinfection by a more virulent strain of the same virus.

It may be concluded that tobacco plants do not recover from ring spot, but enter upon a different phase of the disease following invasion of the embryonic tissue. All new tissue produced by affected plants is susceptible to the virus. There is no conclusive evidence that infection with one strain of the ring-spot virus confers immunity from other strains. The phenomenon can be simply explained by competition between virus strains.

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LITERATURE CITED

1. BLACK, L. M. A study of potato yellow dwarf in New York. Cornell Agr. Exp. Stat. Memoir **209**: 1–23. 1937.
2. JOHNSON, JAMES. An acquired partial immunity to the tobacco streak disease. Wisconsin Acad. Sci. **30**: 27–34. 1937.
3. KUNKEL, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. **24**: 437–466. 1934.
4. PRICE, W. C. Acquired immunity to ring-spot in Nicotiana. Contrib. Boyce Thompson Inst. **4**: 359–405. 1932.
5. ————. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopath. **26**: 503–509. 1936.
6. ————. Specificity of acquired immunity from tobacco ring-spot diseases. Phytopath. **26**: 665–667. 1936.
7. ————. Generalized defense reaction in plants. American Naturalist **74**: 117–128. 1940.
8. VALLEAU, W. D. Seed transmission and sterility studies of two strains of tobacco ringspot. Kentucky Agr. Exp. Stat. Bull. 327, 43–80. 1932.
9. ————. Do tobacco plants recover and develop an immunity from ring spot? (Abstract) Phytopath. **25**: 37. 1935.
10. ————. Do tobacco plants recover from and develop immunity to ringspot? Kentucky Agr. Exp. Stat. Bull. 360, 181–191. 1935.
11. ————, and E. M. JOHNSON. Burning and non-burning strains of tobacco mosaic. Kentucky Agr. Exp. Stat. Bull. 361, 233–238. 1935.
12. WINGARD, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. [U.S.] **37**: 127–153. 1928.

THE INHERITANCE OF RESISTANCE TO POWDERY MILDEW (ERYSIPHE CICHORACEARUM) IN LETTUCE

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(Accepted for publication November 1, 1940)

INTRODUCTION

As one unit in a general program of investigation into the pathologic and genetic problems of lettuce, the organism causing powdery mildew (*Erysiphe cichoracearum* DC.) of lettuce and the inheritance of resistance to the disease have been studied. Powdery mildew seems to be of no great economic importance on outdoor lettuce; but, according to Thompson,[1] may cause considerable damage in the greenhouse, and there the use of resistant varieties is advised.

Previous reports of the occurrence of *Erysiphe cichoracearum* on *Lactuca scariola* L. and *L. sativa* L. (the species used in this study) are fragmentary and in the absence of perithecia the fungus may have been confused with other powdery mildews. Salmon,[2] in 1900, recorded it on several species of *Lactuca.* Seymour[3] (1929) listed many species of *Lactuca* as susceptible but stated that it had not been reported on *L. scariola* in North America. Hashioka[4] has described *Sphaerotheca fuliginea* var. *humili* (Schl.) Salmon on *L. indica* var. *dragoglossa.* For these reasons the vegetative and fruiting characteristics of the organism used in this study are recorded in detail.

Several collections of wild lettuce (*Lactuca scariola*) used in this study have yielded progenies that are homozygous for susceptibility to powdery mildew, while a majority of the cultivated varieties of lettuce (*L. sativa*) tested have been resistant to this disease. Crosses have been made between plants of the susceptible wild lettuce mentioned above and the resistant cultivated variety, Imperial 850. These crosses have provided data for an analysis and interpretation of the inheritance of resistance to the strain of powdery mildew found in Southern California.

It is the purpose of this paper to describe this collection of *Eryisphe cichoracearum* on *Lactuca scariola* and to present an analysis of the inheritance of resistance to the disease in *L. scariola* × *L. sativa* crosses.

MATERIALS AND METHODS

Plants to be tested for their reaction to powdery mildew were inoculated in a reasonably air-tight, glass-sash chamber, constructed for this purpose.

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[1] Thompson, R. C. Improvement of salad crops. U. S. D. A., Yearbook, p. 326–339. 1937.

[2] Salmon, E. S. A monograph of the Erysiphaceae. Torrey Bot. Club Mem. 9: 1–259. 1900.

[3] Seymour, A. B. Host index of the fungi of North America. 732 p. Harvard University Press (Cambridge). 1929.

[4] Hashioka, Y. Specialization in *Sphaerotheca fuliginea* (Schlecht.) Poll. Ann. Phytopath. Soc. Japan 8: 113–123. 1938.

The interior of the chamber was sprayed with water, and, after placing the plants in it, they were inoculated by atomizing a spore suspension in water on both leaf surfaces. This appeared to be the best method for obtaining a uniformly severe epiphytotic.

As a rule the plants remained in the chamber 24 to 48 hours. Partial shading, and a temperature of about 20° C. during inoculation and incubation seemed to produce maximum disease development. In many cases the inoculation was repeated to insure the development of the disease on all susceptible plants.

Microscopic observations of the organism were made on the leaves *in situ* or in water mounts.

The plants of *Lactuca scariola* used in these experiments were grown from seed obtained from a single plant collected near Boulevard, California, at an elevation of about 3000 feet. These plants and progeny from them have been under observation over a period of three years. During this time they have proved to be very uniform in all morphological characters. Following is a description of typical plants:

Leafy stemmed annuals; erect, often branching from the base; 4 to 5 feet high; stems and mid-veins on dorsal surface of leaves hirsute; leaves oblongate, unlobed; no pigment in either stems, leaves, or involucre; ligules yellow; achenes small, dark-brown. Repeated tests over a period of three years gave evidence that plants from this collection and their progeny are homozygous for susceptibility to powdery mildew.

Imperial 850, used as the immune parent, is a crisp-leaf type of lettuce, grown in a limited way for the summer or spring planting in certain of the lettuce producing regions. During repeated tests it has been shown to be homozygous for resistance to this strain of *Erysiphe cichoracearum.*

EXPERIMENTAL RESULTS

Description of the Disease and Organism

Symptoms. After an incubation period of 6 to 10 days from inoculation the mycelium appears first on the ventral leaf surface as small isolated circular patches 1 to 2 mm. in diameter when first macroscopically visible. Later the powdery tufts of white conidia develop (Fig. 1, A). The mycelium continues to spread until in very susceptible plants it entirely covers the leaf (Fig. 1, B). During the spread of the organism the leaf tends to curl either downward or upward at the margin. As the disease becomes more severe the leaf turns yellow (compare with Fig. 1, C). Finally it becomes brown, withers, and dies, but continues to cling to the stem. In older plants the seed stalk is attacked; however, injury from this source seems negligible.

Organism. The white persistent mycelium is confined largely to the ventral leaf surface, a position which seems characteristic for *Lactuca scariola* whether the disease develops in the field or in the greenhouse. Shortly after the mycelium becomes macroscopically visible, conidia (Fig.

2, A) are produced. To aid investigators who are attempting to classify biologic races of *Erysiphe cichoracearum* on the basis of conidial size, spore measurements are shown in table 1. The conidia are hyaline and usually elliptic, though sometimes almost dolioform.

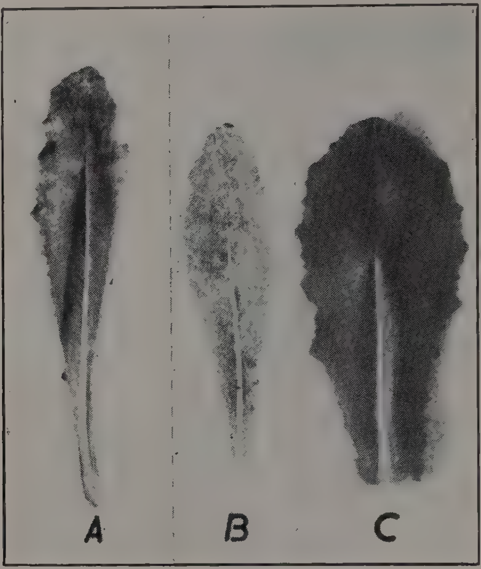

FIG. 1. *Erysiphe cichoracearum* on leaves of *Lactuca scariola*. A. Colonies with conidia just appearing. B. Conidia and mycelium entirely covering leaf. C. Healthy leaf.

TABLE 1.—*Measurements of the reproductive structures of Erysiphe cichoracearum*

Organ or plant part	Number measured	Range		Mean	
		Length	Width	Length	Width
		μ	μ	μ	μ
Conidia	300	33.8– 63.8	18.8– 31.9	45.38[a]	25.88[b]
Perithecia	4	124.0–169.0	124.0–169.0	137.2	137.2
Cells in perithecia	9	11.0– 30.0	8.0– 26.0	17.6	13.0
Asci	9	64.0– 86.0	34.0– 49.0	77.3	42.6
Stalk	5	9.4– 15.0	....	11.3	....
Ascospores	16	30.0– 45.0	15.0– 26.0	34.9	19.1

[a] Standard deviation ± 4.767.
[b] Standard deviation ± 2.918.

Upon many hosts of *Erysiphe cichoracearum* perithecia seldom develop, making positive identification of the fungus impossible. Four mature sexual fruiting bodies and several more immature ones were found on *Lactuca scariola* grown in a greenhouse during the early summer of 1940. A period of moderate temperature with overcast skies preceded their appearance. The perithecia (Fig. 2, B, C, D) are globose and gregarious, arising near a large vein or near the midrib. The numerous, long, septate appendages are densely intertwined, vaguely branched, and are distinctly brown

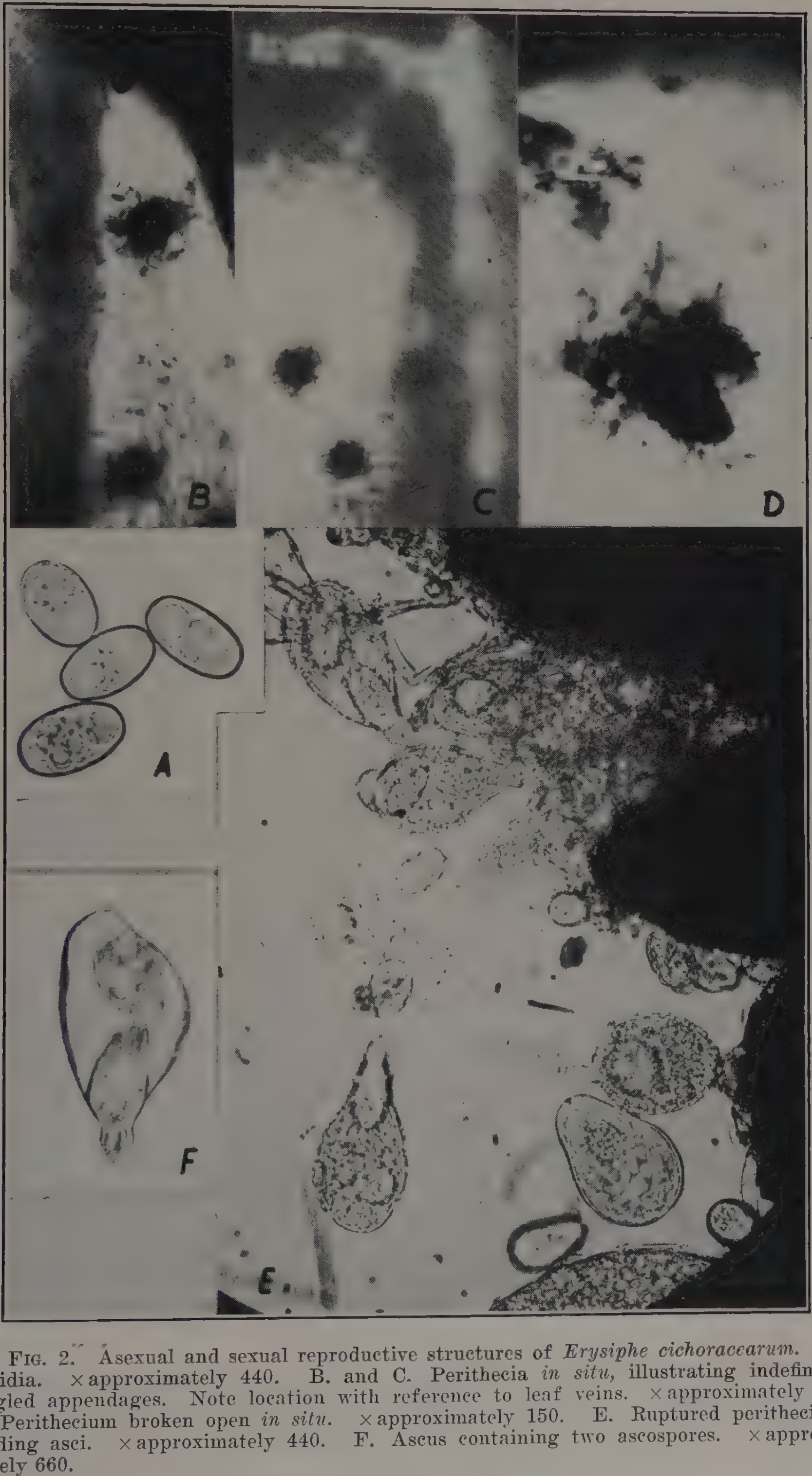

FIG. 2. Asexual and sexual reproductive structures of *Erysiphe cichoracearum*. A. Conidia. × approximately 440. B. and C. Perithecia *in situ*, illustrating indefinite, tangled appendages. Note location with reference to leaf veins. × approximately 50. D. Perithecium broken open *in situ*. × approximately 150. E. Ruptured perithecium exuding asci. × approximately 440. F. Ascus containing two ascospores. × approximately 660.

adjacent to the fruiting body, but lighter near the tip. The size is variable (Table 1). The cells also vary considerably (Table 1), but are quite distinct. There are several hyaline, stalked asci (Fig. 2, E; Table 1) per perithecium and they are elliptic to irregularly elliptic in shape. Each mature ascus contains two ovate to ellipsoidal ascospores (Fig. 2, F; Table 1).

Inheritance of Resistance

Crosses were made using Imperial 850 as the maternal parent. The F_1 plants from this cross were extremely vigorous and typical of first-generation hybrids between cultivated head lettuce and *Lactuca scariola.* The plants formed a thick rosette of basal leaves. The leaves were entire, non-lobate, hirsute along the dorsal surface of the midvein, and light-red along the margin as they became older. There were no indications of head formation, and plants of this progeny produced a seed stem only slightly before the commercial parent.

Of the 10 F_1 plants tested all appeared to be resistant to powdery mildew. Four were saved and the progeny tested to determine their disease reaction. The results are recorded in table 2. These data are reasonably close to expectation, and seem to indicate that a single dominant gene is operating to control immunity.

TABLE 2.—*Segregation of powdery mildew resistance in the F_2 progeny*

Family no.	Total no. of plants	Healthy	Susceptible	Expected healthy	Expected susceptible	χ^2
33591–2	54	46	9	40.5	13.5	2.23
33591–3	33	23	11	24.75	8.25	1.01
33591–4	179	130	49	134.25	44.75	0.36
33591–5	65	43	22	48.75	16.25	2.71
Totals	331	242	91	248.25	82.75	6.31[a]

[a] df = 4; P = 0.2.

Eight F_2 plants were saved for seed, and their progeny tested for reaction toward powdery mildew. The results are recorded in table 3. It was not

TABLE 3.—*Segregation of powdery mildew resistance in the F_3 progeny*

Family no.	Healthy	Susceptible	Total	Phenotype in F_2	χ^2
16964	0	37	37	Susceptible	....
16965	28	9	37	Healthy	0.01
16966	23	4	27	Healthy	1.50
16967	30	7	37	Healthy	0.73
16968	0	35	35	Susceptible	....
16989	25	10	35	Healthy	0.24
33591–2–9	20	15	35	Healthy	5.96
33591–4–2	25	10	35	Healthy	0.24
33591–4–4	35	0	35	Healthy	
33591–4–5	24	11	35	Healthy	0.77
33591–4–7	29	6	35	Healthy	1.05

feasible to make test crosses by mating the F_1 progeny with the recessive parent, since the water technique for depollination is never 100 per cent successful, and the possibility of obtaining self-pollinated individuals instead of cross-pollinated seriously limits the reliability of the data thus secured. This difficulty can be circumvented in certain cases by the use of marker genes, but in the present case marker genes were not available. As a result confirmatory evidence is confined to the F_3 data.

Of the F_3 families tested, 2 proved to be homozygous-susceptible, 1 was homozygous-resistant, and eight were heterozygous (Table 3). With one exception, (Family 33591–2–9) the heterozygous families segregated in the anticipated 3:1 ratio. In this case there is a significant departure from expectation. The nonconformity of this family is not explained, although there is the probability that if larger numbers were tested, the excess of susceptible plants might be overcome. The data of tables 2 and 3 are in substantial agreement and indicate that a single gene is operating to control resistance.

DISCUSSION

The collective species *Erysiphe cichoracearum* is composed of many biotypes. Some investigators[5] have attempted a method of classifying physiologic races of powdery mildews based on the morphological characters of the conidia. Measurements of the conidia from the mildew on *Lactuca scariola* indicate that those of this strain are of unusually large size. They are about 14 μ longer and 10 μ wider than the form Blumer[6] measured on *L. muralis* (L.) Fres. The spores are 10 μ longer and 6 μ wider than those from the race on *Hieraceum pratense* Tausch., which was the largest reported by Blumer. Too few of the other fruiting structures were available for measurement to draw conclusions, but, in general, the organs fall within the range given by Salmon.[7] The characteristic perithecial appendages and the occurrence of more than one 2-spore ascus per perithecium definitely identify the fungus as a race of *E. cichoracearum.*

The elapsed time from incubation until the first appearance of symptoms seems dependent somewhat upon the host reaction, as well as upon the environmental conditions. It appears that there may be genes, other than the one controlling a positive or negative disease reaction, that are active in determining the degree of resistance exhibited by different individuals. That some plants are much more susceptible than others is indicated by the length of the incubation period and by the number, size, and degree of sporulation of the colonies. Some individuals have a few colonies producing conidia, while others may have a similar number of smaller colonies in which sporulation is suppressed. For these same plants the incubation period may vary considerably.

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[5] See footnotes 4 and 6.

[6] Blumer, S. Die Formen der *Erysiphe cichoracearum* DC. Centralblatt. für Bact., Parasitenkunde u. Infektionskrankheiten. **57**: 45–60. 1922.

[7] See footnote 2.

SUMMARY

The occurrence of powdery mildew (*Erysiphe cichoracearum*) on *Lactuca scariola* and *L. sativa* is recorded. The vegetative and reproductive structures of the fungus are described in detail. The discovery of perithecia, with associated reproductive structures, has permitted positive identification of this organism.

Strains of lettuce homozygous for susceptibility and for immunity from powdery mildew were isolated. Appropriate matings, combined with subsequent analysis of F_2 and F_3 data indicate that immunity is controlled by a single dominant gene.

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TUMORS ON ELM AND MAPLE TREES

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THE ELM TUMOR

Outgrowths on the American elm (*Ulmus americana* L.), large enough to be classed as tumors, are not so prevalent as tumors on oaks; and, up to the present time, the disease has not become a rapidly spreading one in the locality where it occurs or throughout the country.

DESCRIPTION OF THE GALLS

The elm tumor is a dark, flat, nodular outgrowth occurring on the trunk or on the roots near the surface of the ground (Fig. 1, A). It has some resemblance to crown gall and usually is so designated. In size it may be a foot or more in diameter, depending on the size of the tree and age of the tumor. It is usually of a slow growing type. Small galls ¼ in. to 2 in. in diameter may occur on the smaller branches, as in the oaks; according to the writer's observations, however, this condition is not a common one.

The term canker is sometimes used loosely, as synonymous with galls and tumors. The two need not be confused, for with cankers there is usually a definite killing of the bark accompanied by sloughing, along with distortion of the trunk. The somewhat rounded outgrowths taking place in tumor formation maintain the live bark-covering until the whole tumor dies, with the possible exception of tumors of immense size.

EARLIER REPORTS OF ELM TUMOR

Emery[1] states that an elm tumor in France is caused by the crown-gall organism, *Bacterium tumefaciens,* that he isolated that organism from elm tumors, and, by inoculating with it, produced two galls on a young elm tree from which the organism was later reisolated.

In 1938, the writer, in an article on tumors of the oak and hickory trees[2] mentioned having worked with elm and maple tumors and stated that fungus-producing pycnidia with phoma-like spores had been isolated from both types but that neither isolation produced infection. A further statement was made that, later, *Phomopsis* spores of the α-type developed in the elm-tumor cultures.

ELM TUMOR STUDIES

Attempts to Isolate Bacterium tumefaciens

The European pathologist probably was right and the elm tumor with which he was working was crown gall. The writer has never been able to isolate the crown gall organism from galls occurring on elm in the United

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[1] Emery, M. Origine bactérienne des tumeurs de l'orme. Comptes Rendus Acad. des Sciences, 193: 76–77. 1931.

[2] Brown, Nellie A. The tumor disease of oak and hickory. Phytopath. 28: 401–411. 1938.

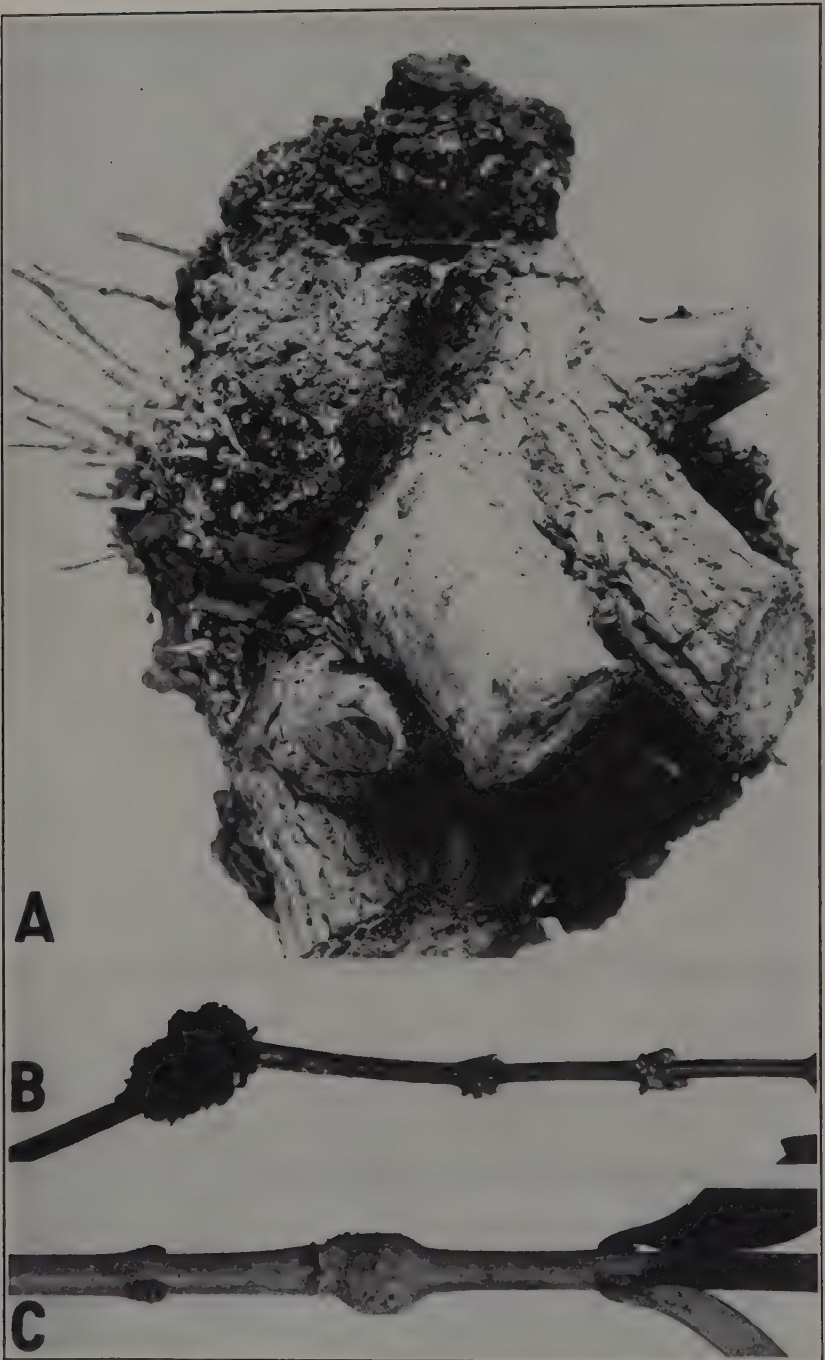

FIG. 1. A. Tumor on American elm at crown from which *Phomopsis* was isolated. The projecting roots developed in a moist atmosphere. Less than ×1. B. Tumor on jasmine produced by inoculating with the elm *Phomopsis*, August, 1939; photographed August, 1940. C. Tumor on privet produced by inoculating with elm *Phomopsis*, August, 1939; photographed August, 1940. B and C. ×1.

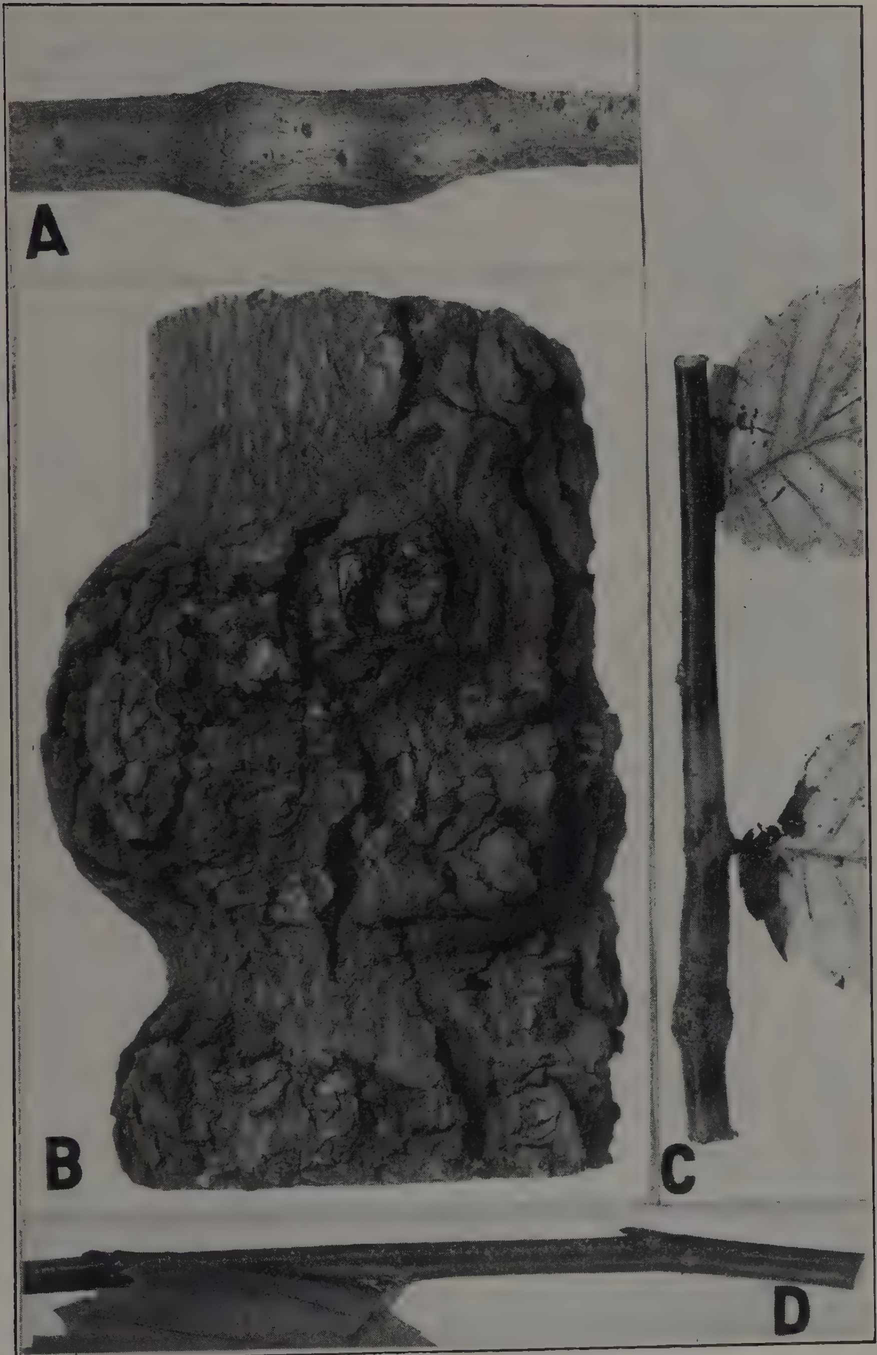

FIG. 2. A. Young stage of maple tumor from Ohio. B. Older stage of Ohio maple tumor from which *Phomopsis* was isolated. C. Tumor on elm stem produced by inoculating with elm *Phomopsis,* March 14, 1940; photographed Sept. 16, 1940. D. Elm stem punctured with sterile needle for control, March 14, 1940; photographed Sept. 16, 1940. All × 1.

States. More than 200 plates have been poured in endeavors to isolate *Bacterium tumefaciens* from elm galls, without success. In this country, *Bact. tumefaciens* does not seem to be the organism actively engaged in that capacity.

A FUNGUS APPEARS TO BE THE CAUSE OF THE DISEASE

No fruiting bodies were seen on the surface of the elm galls nor did any develop when the galls were held for a time in a moist chamber. The failure, however, to isolate the crown-gall organism from the elm tumors and the consistent appearance of pycnidia with phoma-like spores in the cultures made from tissue plantings and from poured plates of elm galls, indicated a fungus as a cause of the disease. Several of the elm isolations were chilled for several months, after which *Phomopsis* spores developed. Sometimes the galls were kept at 10° to 12° C. for a few months, and *Phomopsis* spores of types *a* and *b* were obtained readily in cultures made from isolations of the refrigerated galls. In studying microsections of the tumors, fungus hyphae could be seen in the tissue.

INOCULATIONS WITH PHOMOPSIS CULTURES

The *Phomopsis* cultures, inoculated into the nodes of American elm trees produced slow-growing tumors from which the *Phomopsis* was reisolated. The galls did not begin to develop until a month or more after inoculation, and in 6 months were no larger than 7–10 mm. (Fig. 2, C) in diameter. Compared with the speed of *Bacterium tumefaciens,* which produces a 1.5 in. or larger tumor on daisy or marigold in 3 months or less, and on peach trees $\frac{1}{2}$ to $\frac{5}{8}$ in. tumors in less than 2 months, the elm outgrowth is a very slow-growing one. The same slow-growing tumors were produced with the elm *Phomopsis* on privet (*Ligustrum vulgare*), *Jasminum nudiflorum* (Fig. 1, C and B), and *Viburnum opulus* from which the fungus was reisolated. These various hosts were used for inoculations in conjunction with the elm trees, as *Phomopsis* tumors occur naturally on them.[3,4]

As an example of the slow growth of this type of tumor, it took over 5 years for the privet *Phomopsis* to produce tumors $1\frac{1}{2}$ in. in diameter on privet stems. During this time the plants were growing in the greenhouse under very favorable conditions.

BACTERIUM TUMEFACIENS PEACH STRAIN INOCULATED INTO ELMS

Not being able to isolate *Bacterium tumefaciens* from elm tumors, it was thought crown gall could, perhaps, be produced on elm trees by inoculating with a strain of *Bacterium tumefaciens.* Out of 49 inoculations made into elms with a virulent strain, two galls formed. These were produced on young growth of 3- to 4-year-old seedlings that was tender and kept so by pushing the trees into a fairly long period of rapid growth. The other trees

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[3] Brown, Nellie A. A fungus gall on *Viburnum* mistaken for crown gall. Phytopath. **24**: 1119–1120. 1934.

[4] ———. Privet and jasmine galls produced by a species of *Phomopsis.* Phytopath. 26: 795–799. 1936.

in the experiment, likewise stimulated to continued growth, failed to produce any sign of a tumor. The two galls noted were produced by the peach strain. These galls were of a rounded type and did not simulate the rather flat tumors that usually occur on the elm. They were 7 and 10 mm. in diameter in 4 months, but did not grow any larger within the 6 months they were under observation.

It is not unlikely that in nature, under conditions and in localities favorable for them, *Phomopsis* and *Bacterium tumefaciens* are able separately to produce outgrowths on the elm tree. As tumors on elm are not produced readily with either organism, and less readily with the bacterium, it may be said that both organisms are weakly parasitic on the elm. Of 106 inoculations into elm trees with elm *Phomopsis,* after 6 months only 31 produced galls; there were swellings on 24 others, indicating that infection had started, and 41 showed no indication of infection. The *Phomopsis* was reisolated from some of the galls and swellings.

TABLE 1.—*Inoculations into various hosts with the elm Phomopsis*

No. of inoculations into		No. of galls	No. not infected
Privet out-of-doors and in greenhouse	60	18	42
Viburnum	5	4	1
Jasmine	8	5	3

NO PERFECT STAGE OF THE FUNGUS WAS FOUND

Hundreds of cultures of the elm fungus growing in different kinds of media were examined, but the perfect stage of *Phomopsis* had not formed, nor was it found in or on the tumors of different ages. Oatmeal-agar cultures, 3 to 7 months old, often had blackened, dried, slightly bent strands of mycelium resembling perithecial necks. These strands were connected with an undifferentiated stroma showing no evidence of the formation of perithecia.

Up to the present the *Phomopsis* has not been assigned to any particular species.

THE MAPLE TUMOR

Description of the Disease

The maple tumor has been noted on *Acer rubrum* L. and on *A. saccharum* Marsh, by the writer perhaps more than on other species. It occurs in the United States less frequently than does the elm tumor, and is seen in the Lake States and the North more than in the South. In young stages the appearance of the disease is one of smooth swellings (Fig. 2, A, and Fig. 3, A) or knots (Fig. 3, B), unlike the young tumors of oak, hickory, privet, viburnum, and others. In older stages the smooth swellings become rough and appear somewhat cankerous (Fig. 2, B, Fig. 3, C). When they cover the entire limb, as they sometimes do, the latter eventually dies.

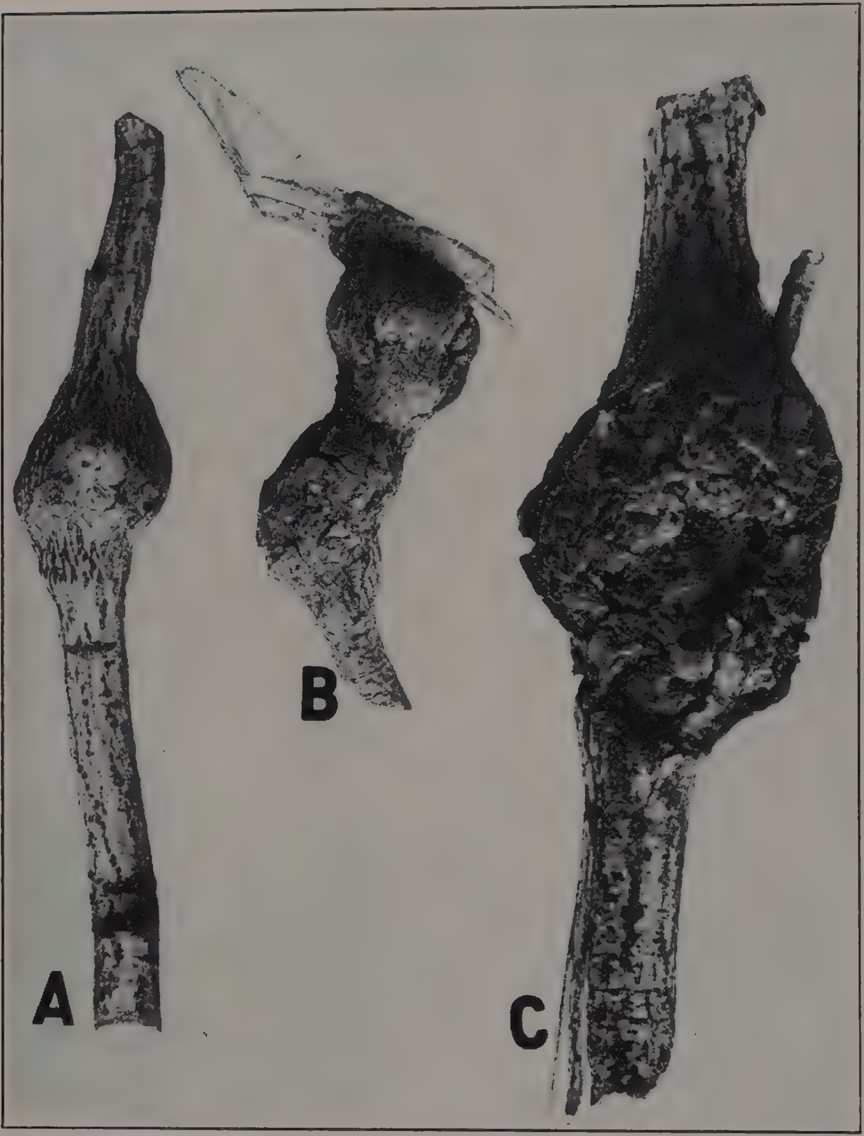

FIG. 3. Different stages of development of maple tumor occurring in Michigan. Phoma-like spores were isolated from B. All tumors × 1.

EARLY WORK ON THE MAPLE TUMOR DISEASE

The early work on maple tumors was done in 1920–1921 and was mentioned in a paragraph in the paper entitled "The Tumor Disease of Oak and Hickory Trees."[5] The 36 plates poured from Michigan maple tumors in 1920 gave no crown-gall colonies. No fruiting bodies were seen on the surfaces of the tumors, but, from tissue plantings on various media, a fungus appeared repeatedly that produced pycnidia with phoma-like spores. Cultures of this fungus were inoculated into 3 different species of maple trees in Washington, D. C., by the writer and in Michigan by Winifred E. B. Chase. A total of 72 inoculations was made but no tumors resulted, only swellings, or there was no reaction whatever. Dr. Chase sent some of her inoculated stems and some of the controls to show that her results duplicated those made in Washington, which were considered practically negative.

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[5] See footnote 2.

Microsections made through some of the inoculated areas showed that the fungus embedded in the tissue had produced pycnidia with tiny phoma-like spores. The inoculations had been made nearly two months earlier. The significance of this fruiting body was not suspected and the cultures were discarded as the wrong pathogen.

WORK WITH THE MAPLE TUMOR IN 1940

In the spring of 1940 some maple galls from Ohio were received from the Division of Forest Pathology, U. S. Department of Agriculture, with the request to ascertain the cause, if possible. Isolations were made from young outgrowths and later older ones to attempt the recovery of *Bacterium tumefaciens.* It could not be isolated from either young or older material.

ISOLATION OF A FUNGUS

No fruiting bodies were on the surface of any of the galls received. Tissue plantings on different media from the older galls gave pycnidia containing *Phomopsis* spores of both *a* and *b* types. Phoma-like spores were not observed. Sterile tap water in tubes was the best medium to start the growth of the fungus from the tissue plantings; the fungus was then transferred to oatmeal and corn-meal agar on both of which it fruited. Attempts to isolate *Phomopsis* from the very young maple tumors were unsuccessful.

As with the elm *Phomopsis,* the maple form has not been assigned to any species.

TESTS WITH THE MAPLE PHOMOPSIS

Unfortunately, the work with the maple tumor began so late in the spring of 1940 that by the time the cultures were obtained and ready to use it was too late to get young maple trees for inoculation purposes, and none were otherwise available. Inoculations, however, were made into privet stems in the greenhouse. On this host small galls had started to grow in 2½ months.

NOTES ON THE EARLY STAGES OF PHOMOPSIS TUMORS

The initial stages of the elm and maple *Phomopsis* tumors do not resemble those of the oak and hickory. The elm usually bears a flat mass of root primordia, and the maple shows smooth swellings. The earliest stage of an oak or hickory tumor resembles a small canker. This develops into a gall in the course of a year or two. As the majority of the other *Phomopsis* galls studies so far, viburnum, privet, coralberry, forsythia, blueberry and jasmine usually begin with a mass of root primordia, the large, definitely smooth, swelling of the maple tumor suggests another cause. And it is possible that the beginning of the maple tumor may be due to a root obstruction or growth disturbance, a food unbalance, or other cause. Into this environment the *Phomopsis* can establish itself and develop a tumor. It will take more observations and research to learn the whole story of the maple tumor. However, the smooth surface of an outgrowth must not be considered too significant, as the elm *Phomopsis,* inoculated into privet, produced a smooth type of gall that remained so when it had become a half inch and more in diameter. Inoculations with *Bacterium tumefaciens* occasionally produce a smooth type of gall, depending on the kind of tissue infected and the rate of tumor growth.

MOISTURE AND THE CONTROL OF THE ELM AND MAPLE TUMORS

Moisture seems necessary for *Phomopsis* to establish itself in the stem tissue and produce a tumor. The pathogen enters the stem through some opening, as a wound or otherwise, and if moisture is lacking because of heat, drying winds, or drought, the infection fails to take. Many of the inoculations made on elm stems no doubt failed because of insufficient moisture. This factor may be used in preventing the spread of the disease on a tree, or trees, in the vicinity where the disease occurs. The close planting of trees and shrubs helps retain moisture and should be avoided where elm and maple tumors occur. All the tumors that can be cut out should be removed and burned.

SUMMARY

Bacterium tumefaciens was not isolated from either the elm or maple tumors.

A species of *Phomopsis* was isolated from tumors on either host and the disease was reproduced by inoculations with both isolations. The *Phomopsis* from either host has not yet been assigned to any particular species.

Neither disease is of a rapidly spreading or dangerous type, but it can badly disfigure a tree and eventually kill it.

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A SOLUTION–CULTURE INFECTION METHOD USED IN THE STUDY OF FUSARIUM WILTS[1]

G. M. Armstrong

(Accepted for publication October 8, 1940)

In studying the mineral nutrition of cotton plants grown in solution cultures, it was desired to use plants infected with *Fusarium vasinfectum* Atk. Difficulties were frequently experienced, however, in obtaining high percentages of infection of seedlings grown in naturally or artificially infested soil before transferring them to the solution cultures. Attempts were made, therefore, to secure a method that would provide consistently high percentages of infection and also simplify the production of seedlings. The methods reported herein originated from the efforts to reach these objectives.

The nutrient solution in which all the plants were grown had the following composition: 0.002 M magnesium sulphate; 0.005 M calcium nitrate; 0.002 M potassium sulphate; 0.002 M primary ammonium phosphate; traces of iron citrate and of other minor elements: zinc, boron, manganese, and copper. The pH of the solution made up with distilled water was about 5.1. The solution was replaced every 7 to 10 days and distilled water was added between changes to replace water lost by transpiration and evaporation.

The culture solution for the fungus contained the same salts as the solution used for the plants, except that the concentration was doubled and glucose was added at the rate of 20 grams per liter. Before inoculation, the fungus cultures were diluted by adding an equal volume of distilled water (a dilution of 1 to 4 has been successfully used on one occasion). The fungus was grown for 3 days in 1 to 3-liter Erlenmeyer flasks with 400 ml. of solution per liter flask and correspondingly increased amounts in the larger flasks. The flasks were agitated frequently during growth in order to obtain a finely divided suspension of fungus material. The short period of exposure of the roots to a fresh fungus suspension practically excludes any toxic effect of staling products produced during growth.

METHODS EMPLOYED TO OBTAIN SEEDLINGS

First Method

In the first infection experiments, acid-delinted seed were germinated in trays of sterilized sand and the most vigorous seedlings were then transplanted to solution cultures in the manner described below. The root systems of seedlings grown as above were dipped into nutrient solution cultures of the pathogen for periods varying from 10 to 90 minutes.[2] The seedlings were then transferred to the regular nutrient solutions.

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[1] Technical Contribution No. 77 from the South Carolina Agricultural Experiment Station. Cooperative Investigations with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

In another early study concerned with the pathogenicity of several isolates of *Fusarium vasinfectum* Atk., seedlings were germinated in trays with moist paper towels and thereupon transferred to a nutrient solution in glazed ½-gal. crocks for several days before inoculations were made. The crocks were covered with paraffined wooden tops having eight ½-in. holes in which the seedlings were held by small pieces of nonabsorbent cotton. In this experiment the roots were allowed to stand in the nutrient solution culture of the pathogen for 30 minutes and then were returned to the glazed crocks.

The experiment (Table 1) was conducted during the summer in a greenhouse at Clemson, South Carolina,[3] by the method just described. The average temperature was in the range of the optimum for cotton-wilt infection (25–30° C.). The degree of virulence of the 3 fungus isolates was indicated as very strong (No. 4), strong (No. 6), and weak (No. 3) from soil pot experiments.[4]

The data of table 1 show that these isolates in solution culture exhibited the same relative degrees of virulence as in the soil experiments. All plants were examined at the conclusion of the experiment for discoloration of the xylem. Reisolations of the fungus were attempted by cutting about an inch section from the lower part of each stem, surface-disinfecting it with 3 per cent sodium hypochlorite, cutting it lengthwise, and placing the portions upon acid potato-dextrose agar in Petri dishes.

The high percentage of recoveries, even of the weak isolate of the pathogen from varieties of cotton as resistant as Sea Island, indicates that the solution-culture method is a very effective means of producing infection. It appears to correspond to severe wilt conditions in the field.

Broadfoot and Tyner[5] have developed a somewhat similar procedure for producing wheat seedlings and infecting them with foot-rot organisms and it is of interest that the two investigations were proceeding simultaneously, each without the knowledge of the other. They mention several of the difficulties with soil inoculations. The methods for securing infections with the fusarium wilt of cotton were developed in 1937 and 1938. The first method was tedious since it involved planting the seed in sand or in trays with moist paper towels, placing each seedling in a hole in a container, and packing cotton around each seedling for support.

Second Method

A second method, that of the tank or tray culture, was next tried in an

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[2] The first experiments were done by Dr. N. A. Schappelle in 1937 after consultation with the writer.

[3] This experiment was carried out by Dr. R. Weindling.

[4] Armstrong, G. M., J. D. MacLachlan, and R. Weindling. Variation in pathogenicity and cultural characteristics of the cotton-wilt organism, *Fusarium vasinfectum.* Phytopath. **30**: 514–520. 1940.

[5] Broadfoot, W. C., and L. E. Tyner. Studies in foot- and root-rot of wheat. VI. Methods of securing infection of wheat seedlings for study in nutrient solutions. Canad. Jour. Res. **16**: 253–261. 1938.

TABLE 1.—*Results obtained with solution-culture infection method using seven cotton varieties with three isolates of Fusarium vasinfectum*

Isolate of *F. vasinfectum*	Variety of cotton[a]	Plants 4 weeks old, per cent severely damaged	Disease analysis at conclusion of experiment—plants 7 weeks old				
			Per cent severely diseased	Per cent slightly diseased	Per cent "healthy"[b]		Per cent of plants giving *F. vasinfectum* reisolations
					With vascular discoloration	Without vascular discoloration	
Isolate No. 4	Farm Relief 2	37.5	95.8	4.2	....	....	91.7
	Rowden 2088	20.8	70.8	25.0	....	4.2	79.2
	Coker 100	25.0	54.2	....	25.0	20.8	79.2
	Cook 307	20.8	50.0	16.7	25.0	8.3	41.7
	Miller 610	25.0	45.8	29.2	12.5	12.5	79.2
	Dixie Triumph 12	....	20.8	29.2	33.3	16.7	66.7
	Sea Island	....	....	8.4	45.8	45.8	41.7
Isolate No. 6	Farm Relief 2	20.8	83.3	4.2	4.2	8.3	79.2
	Miller 610	4.2	33.4	25.0	20.8	20.8	66.7
	Rowden 2088	....	29.2	8.3	33.3	29.2	62.5
	Coker 100	....	25.0	12.5	12.5	50.0	70.8
	Dixie Triumph 12	....	20.8	25.0	50.0	4.2	37.5
	Cook 307	....	16.7	4.2	20.8	58.3	62.5
	Sea Island	....	....	....	29.2	70.8	25.0
Isolate No. 3	Rowden 2088	....	4.2	33.3	41.7	20.8	50.0
	Miller 610	....	4.2	....	33.3	62.5	37.5
	Farm Relief 2	....	....	16.7	58.3	25.0	70.8
	Cook 307	....	....	8.3	29.2	62.5	16.7
	Dixie Triumph 12	....	....	4.2	8.3	87.5	8.3
	Coker 100	....	....	....	37.5	62.5	37.5
	Sea Island	....	....	....	16.7	83.3	37.5

[a] Twenty-four plants of each variety were inoculated with the isolate indicated.
[b] No external symptoms discenible.

effort to simplify the production of seedlings for inoculations. This method has been used in several experiments during the past 2 years. Some metal trays (Fig. 1) 21″ × 13″ × 8″, which were used in the laboratory, have been found very satisfactory for growing cotton, tobacco, snap beans, cowpeas, and *Cassia tora* L., all of which grow very well for 8 or 9 weeks without aeration of the culture solution. A wooden frame with galvanized hardware cloth of $\frac{1}{4}$-in. mesh attached to the lower inner edge was fitted tightly into the tray to a depth of an inch. Tray, frame, and hardware cloth were all coated with paraffin of 60° C. melting point. Excelsior, which had been boiled and soaked in water for 3 to 4 hours, was placed over the hardware cloth of the frame to serve as a seed bed. Soaked seed of the above-mentioned plants, except tobacco, were planted in the excelsior and then kept covered with moistened toweling until the roots had grown into the water or nutrient solution below. The water level in the tray was kept in contact with the hardware cloth until the roots were about 2 inches long, when the level was lowered about 1 inch. This procedure gave better aeration and also retarded the development of molds in the excelsior plant bed.

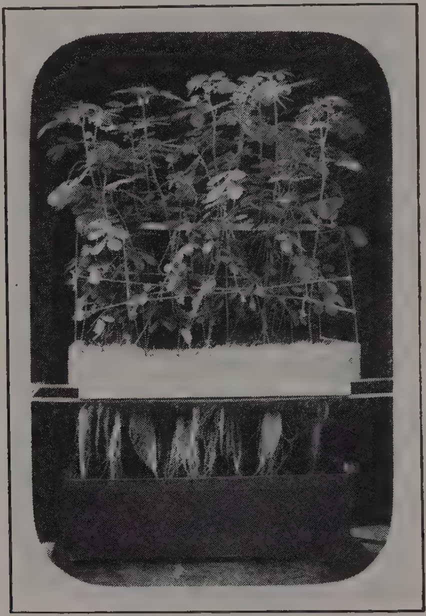

FIG. 1. Equipment employed in growing cotton in culture solutions.

Very small seed, *e.g.*, tobacco, cannot be planted directly in the excelsior, so these were planted in soil flats, the seedlings later being transplanted to the trays.

Another tray only 3 in. deep, but otherwise like the regular culture tray, was used for the inoculations because a smaller quantity of inoculum was needed to fill it. Inoculations were made by dipping the roots in a nutrient solution culture of the pathogen for 10 minutes and then returned to the usual nutrient solutions. Experience has shown that exposure of the roots to the inoculum for 10 minutes gave from 80 to 100 per cent infection with susceptible strains of cotton, tobacco, or cowpeas. The inoculum contained a moderately dense mass of filaments and spores of the fungus; and it is possible that merely dipping the roots would have sufficed. As an example of the results of one of many experiments with the trays, 67 plants of a susceptible variety of cotton were inoculated on April 25, 1939, and all plants remaining alive were removed 9 weeks later. Ninety-one per cent of them showed external symptoms of wilt during the course of the experiment and the fungus was recovered from 78.7 per cent of these plants. In the most recent experiment, 100 per cent of the plants of the same variety, inoculated with the same isolate of the fungus, showed external symptoms of wilt.

The tank or tray method described allows the use of a large number of plants per unit area, especially of susceptible varieties, since wilting begins in 9 to 12 days after inoculation and plants can be gradually removed. Either 75 to 90 cotton plants or 25 tobacco plants have been grown per tray.

During the summer months, definite differentials of disease resistance have been obtained between susceptible and tolerant cotton varieties within a growth period of 5 to 7 weeks, whereas a considerably longer time is required when the plants are growing in soil. During the winter, the time required has occasionally been 8 to 9 weeks.

Aeration of the culture solution may be necessary for some plants, though no benefits from aeration were noted in cultures of cotton, tobacco, and *Cassia tora* L. during the relatively short period of an experiment. In other experimental work with cotton, where plants were grown to maturity in 17-l. Pyrex jars of greater depth than the trays, aeration was necessary for best growth.

This method promises to supplement other methods commonly used to infect plants with pathogens that enter through the roots. Furthermore, it offers a new line of approach for investigating certain phases of fundamental research with soil-borne pathogens. For instance, in work on the nature of disease resistance, the influence of the various soil factors can be excluded while studying infection of the root and development of the disease. A rapid method for testing the resistance of varieties to the wilt disease is afforded. This can be of practical value to the plant breeder, since, in a relatively short time, it is possible to separate promising wilt-resistant progenies from susceptible ones and thereby save extensive field tests.

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THE EFFECT OF LEAF RUST ON THE CAROTENE CONTENT OF WHITE CLOVER[1]

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(Accepted for publication October 20, 1940)

INTRODUCTION

The occurrence of leaf rust (*Uromyces trifolii trifolii-repentis* (Liro) Arth.) on white clover (*Trifolium repens* L.) in pastures and experimental plots has introduced the question of the economic importance of this disease in reducing the yield and nutritive value of the crop. Studies have been undertaken on the effect of this disease on the chemical composition of the host plant.

Results obtained concerning the effect of the rust on the carotene content are reported here. The only comparable researches known to the writers are those of Johnson[3,4] in which leaf-hopper attack reduced materially the carotene content and thus the potential Vitamin A activity of alfalfa.

MATERIAL AND METHODS

Seven susceptible and 2 resistant clones were selected for study in the white-clover nursery that had been planted in 1938. One plant in each row of clonal material was dusted with sulphur and was in proximity to another plant in the same row, which had been left dust-free and free to develop rust. The clonal rows were distributed at random in 3 replications. Dusting was performed 7 times between June 1, 1940, and the last sampling date.

Samples were plucked by hand between July 17 and August 2, 1940. The date of sampling was determined by the spread of the rust. All replications of the same clone were not sampled on the same date but the sulphured plant and the nontreated one in the same row were sampled simultaneously.

The entire leaves, including petioles, were gathered between the hours of 8 and 9 a.m. and brought immediately to the laboratory in closed containers. Five-gram portions of fresh leaves were weighed out for carotene and other portions for dry-weight determinations. The leaves from the sulphured plants were practically free from rust; those from the nontreated plants contained both rusted and non-rusted leaves, according to their occurrence on the plant.

In order to study the differences between rusted and non-rusted leaves from the same plant, 5 bulk samples, each from an unrelated plant, were collected. In the laboratory the leaves from each of these bulk samples were separated into 3 classes, viz., heavily rusted, lightly rusted, and rust-free. Each class was sampled separately.

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[1] Contribution No. 16, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the northeastern States.

[2] Acknowledgments are due to Dr. S. S. Atwood for making available the plant material and to Mr. A. G. Morin for assistance in the analyses.

[3] Johnson, H. W. Effect of leaf-hopper yellowing upon the carotene content of alfalfa. Phytopath. **26**: 1061–1063. 1936.

[4] Johnson, H. W. Further determinations of the carbohydrate-nitrogen relationship and carotene in leaf-hopper-yellow and green alfalfa. Phytopath. **28**: 273–277. 1938.

Immediately after weighing, the leaves were disintegrated by grinding under ethyl alcohol in a Waring Blendor. Potassium hydroxide was added, and the sample was boiled gently for 30 minutes under a reflux. After cooling and decantation the residue was extracted with petroleum ether and the determination was continued by the Guilbert[5] method, as described by Peterson, Hughes, and Payne.[6] Final estimation of β-carotene was made by the KWSZ photometer.

RESULTS

Table 1 gives the data that compare the carotene content of sulphured (non-rusted) with rusted plants. A lower mean carotene content of the rusted plants was found for each of the 7 susceptible clones. The average difference between the two groups was 21.4 per cent. The analysis of variance (Table 2) indicates that highly significant differences were obtained

TABLE 1.—*Carotene content of rusted and sulphured (non-rusted) leaves of seven susceptible clones of white clover*

Clone	Carotene content in parts per million of the dry weight[a]		Percentage decrease in carotene of rusted plants
	Sulphured	Rusted	
1	423	338	20.1
2	385	301	21.7
3	374	316	15.7
4	402	328	18.5
5	438	310	29.3
6	326	284	12.8
7	372	261	30.0
Mean	388	305	21.4

[a] Mean of three replications.

TABLE 2.—*Analysis of variance for carotene content of rusted and sulphured (non-rusted) plants of 7 susceptible clones of white clover*

Source of variation	D/F	Mean square	F value	Value of F for P of	
				.05	.01
Treatments	1	72,027.7	54.76	4.22	7.72
Replications	2	1,570.4	1.19	3.37	....
Clones	6	4,838.9	3.68	2.47	3.59
Treatment × clones	6	1,357.5	1.03	2.47	....
Error	26	1,315.1	....	....	....

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[5] Guilbert, H. R. Determination of carotene as a means of estimating the vitamin A value of forage. Ind. Eng. Chem., Anal. Ed. 6: 452–453. 1934.

[6] Peterson, W. J., J. S. Hughes, and L. F. Payne. The carotenoid pigments. Kansas Agr. Exp. Stat. Tech. Bull. 46: 5–74. 1939.

between rusted and sulphured plants, that replications were uniform, and that differences among clones were significant.

Table 3 contains the data obtained from 2 resistant clones. Rust was not present, but the sulphured plants contained less carotene than the non-treated.

TABLE 3.—*Carotene content of sulphured and non-sulphured leaves of two resistant clones of white clover*

Clone	Carotene content—parts per million of dry weight		Percentage decrease of carotene in sulphured plants
	Sulphured	Not sulphured	
1	465[a]	513[a]	9.3
2	443[b]	498[b]	11.0
Mean	454	506	10.2

[a] Mean of 3 replications.
[b] Mean of 2 replications.
The F value for treatments is 6.88. The F value necessary to give a P of .05 is 5.99.

Table 4 presents the analyses obtained from separated leaves of the same plant. The results show that heavily rusted leaves are on the average 45 per cent lower and that the lightly rusted leaves are 24 per cent lower in carotene than the non-rusted leaves of the same plant.

TABLE 4.—*Carotene content of non-rusted, lightly rusted, and heavily rusted leaves from the same plant in five plants of white clover*

Plant	Carotene as parts per million of the dry weight		
	Non-rusted	Lightly rusted	Heavily rusted
1	410	293	248
2	478	330	210
3	396	313	191
4	413	343	272
5	392	304	226
Mean[a]	418	317	229

[a] Difference of means necessary to give a P of .01 = 58.8.

DISCUSSION

The comparison of rusted white clover plants with adjacent plants protected from rust by sulphur dust showed that the former were lower in carotene content by an average of 21 per cent. That the actual percentage loss of carotene by rust is greater than this figure is suggested strongly by data obtained from resistant clones where no rust was present and where sulphuring lowered the carotene 10 per cent. It is possible therefore that in nonsulphured susceptible plants rust may cause loss of a third of the carotene, even though all leaves may not be infected. Where leaf separations were made on plants under such conditions, an average loss amounting to 45 per cent was recorded in the heavily rusted leaves. The separation of the leaves in this case was based only on rust infection; no consideration was given to the age of the leaves. It is possible that younger leaves that had not built up sufficient inoculum may have been more numerous in the non-rusted group; hence differences may in part be attributable to age.

SUMMARY

Leaves from rusted white-clover plants contained 21 per cent less carotene than adjacent plants protected by sulphur. Sulphured rust-resistant plants contained 10 per cent less carotene than similar nonsulphured plants. Rusted leaves were considerably lower in carotene than rust-free leaves from the same plant.

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INHERITANCE OF RESISTANCE TO STEM RUST IN CROSSES WITH KENYA VARIETIES OF TRITICUM VULGARE VILL.[1]

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(Accepted for publication September 30, 1940)

In recent years considerable attention has been given to the varieties of *Triticum vulgare* Vill. from Kenya colony, East Africa, as sources of stem rust resistance. Five strains of such wheats have been reported as practically immune in all stages of growth from a number of physiologic races of stem rust (*Puccinia graminis tritici* Eriks. and Henn.) in Canada.[2] At the University of Sydney, in collaboration with W. L. Waterhouse, a study was commenced to determine the mode of inheritance of the Kenya type of resistance. Hybrid material obtained from this study was tested at University Farm, St. Paul, Minnesota, as adult plants in the field to a collection of at least 30 physiologic races of *Puccinia graminis tritici* and as seedlings to single races in the greenhouse. Two varieties of Kenya wheat, Kenya 744[3] and Kenya 745, differing in morphological characters, were crossed together and with 2 rust-susceptible Australian varieties, Federation 107 and Dundee 985. A large number of F_3 lines of the cross Federation 107 × Kenya 745 tested as seedlings with races 17 and 56 gave a reaction highly correlated with the reaction of mature plants in the field to a mixture of races. In table 1 are given the results for 1940, and these are essentially the same as those obtained in 1939.

TABLE 1.—*Distribution of 241 F_3 lines of the cross Federation 107 × Kenya 745 for stem rust reaction to a collection of races in the field in 1940 and for seedling reaction to race 56*

Field reaction	Seedling reaction to race 56			
	Resistant	Segregating	Susceptible	Total
Semi-resistant	54	1	–	55
Segregating	3	129	2	134
Susceptible	–	–	52	52
Total	57	130	54	241

P for agreement with a 1: 2: 1 ratio for seedling reaction lies between 0.30 and 0.50.
P for agreement with a 1: 2: 1 ratio for field reaction lies between 0.20 and 0.30.

In the cross Federation 107 × Kenya 745 smaller numbers of F_3 lines whose seedling reactions to races 17 and 56 had been determined were inoculated with races 1, 11, 14, 15, 21, 34, 36, 38, 47, 49, 97, and 147. All these races except race 38 gave a reaction on these seedling F_3 lines corresponding to that given by these same lines when tested as mature plants to a collection of races in the field. Race 38 was not common in the nursery in either 1939 or 1940 when these tests were carried out. Determinations of physiologic races from the collections made in the breeding nursery indicated that in 1939 the most common races involved in the epidemic were 11, 17, 34, 36, 47, and 56, while, in 1940, races 15, 17, 34, 36, 49, 56, and 147 predominated. Seedlings of Federation 107 are moderately resistant to race 38 and from inoculations of 85 F_3 lines it was found that the same gene in Federation 107 governs susceptibility to race 56 as governs moderate resistance to race 38. This gene is allelomorphic to the gene in Kenya 745, which is responsible for resistance to races 1, 11, 14, 15, 17, 21, 34, 36, 38, 47, 49, 56, 97, and 147.

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[1] These studies were commenced at the University of Sydney, New South Wales, and were continued in the Division of Plant Pathology and Botany of the University of Minnesota while the writer was Thomas Lawrence Pawlett Scholar of the University of Sydney.

[2] Peterson, R. F., T. Johnson, and Margaret Newton. Varieties of *Triticum vulgare* practically immune in all stages of growth to stem rust. Science **91**: 313. 1940.

[3] Varieties carry the Sydney University Accession Number.

The cross Federation 107 × Kenya 744 was tested in 1940 and the segregation for seedling reaction to race 36 and for mature plant reaction in the field is given in table 2. From these figures and from those given in table 1 it seems that both resistant Kenya varieties have a single factor for resistance to stem rust. A similar result was obtained when Dundee 985 was used as the susceptible parent. A study of the F_1 plants of each of these crosses showed that resistance is only partially dominant.

TABLE 2.—*Distribution of 205 F_3 lines of the cross Federation 107 × Kenya 744 for stem rust reaction to a collection of races in the field in 1940 and for seedling reaction to race 36*

Field reaction	Seedling reaction to race 36			
	Resistant	Segregating	Susceptible	Total
Moderately resistant	56	5	–	61
Segregating	4	85	8	97
Susceptible	–	5	42	47
Total	60	95	50	205

P for agreement with a 1: 2: 1 ratio for seedling reaction lies between 0.30 and 0.50.
P for agreement with a 1: 2: 1 ratio for field reaction lies between 0.20 and 0.30.

The figures presented in table 3 indicate that in a cross between the two Kenya varieties a susceptible class was obtained when seedlings of F_3 lines were inoculated with race 17. This can be explained on the basis that the factors for resistance to race 17 in Kenya 744 and Kenya 745 are independently inherited.

TABLE 3.—*Observed and calculated ratios of F_3 lines of the cross Kenya 745 × Kenya 744 for seedling reaction to race 17 of stem rust*

	Resistant	Segregating	Susceptible	Total
Observed	142	178	22	342
Calculated	149.62	171.00	21.38	342

P for agreement with a 7: 8: 1 ratio lies between 0.30 and 0.50.

During the 1940 season at St. Paul some wheat varieties were less resistant than in the previous year. Varieties and lines that, in 1939, were classed as resistant with a trace to 15 per cent of stem rust, had up to 35 per cent in 1940 and were classed as semi-resistant. At University Farm, in 1938, Kenya 744 was more resistant than Kenya 745.[4] This was also true in 1939 and 1940. In 1939 these were both classed as resistant with a trace and a trace to 15 per cent stem rust, respectively, while, in 1940, they were classed as moderately resistant and semi-resistant respectively.

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[4] Unpublished results of cooperative investigations between the United States Department of Agriculture, Division of Cereal Crops and Diseases, and the Minnesota Agricultural Experiment Station.

PHYTOPATHOLOGICAL NOTES

Further Notes on Fig Mosaic.—Eight years ago the writers presented[1] an account of a fig mosaic in California. Apparently this was the first published account of this virus disease, which, in California, is almost universal on fig trees. Various other observers have since attested to the presence of fig mosaic in widely separated countries. In the meantime some new manifestations of the disease have shown up in California, which we wish to note here along with certain other leaf troubles that might be confused with mosaic.

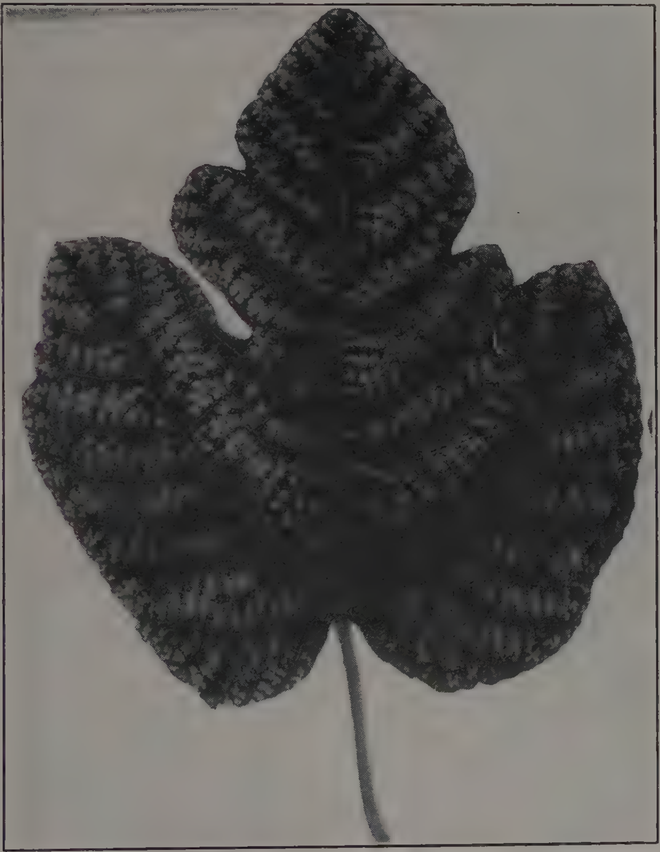

FIG. 1. This leaf of a seedling fig probably shows a deficiency disease rather than a mosaic.

The list of references to the occurrence of fig mosaic is too long to include in this short note. Suffice it to state that leaf mosaic of the fig has been

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[1] Condit, Ira J., and W. T. Horne. A mosaic of the fig in California. Phytopath. **23**: 887–896. 1933.

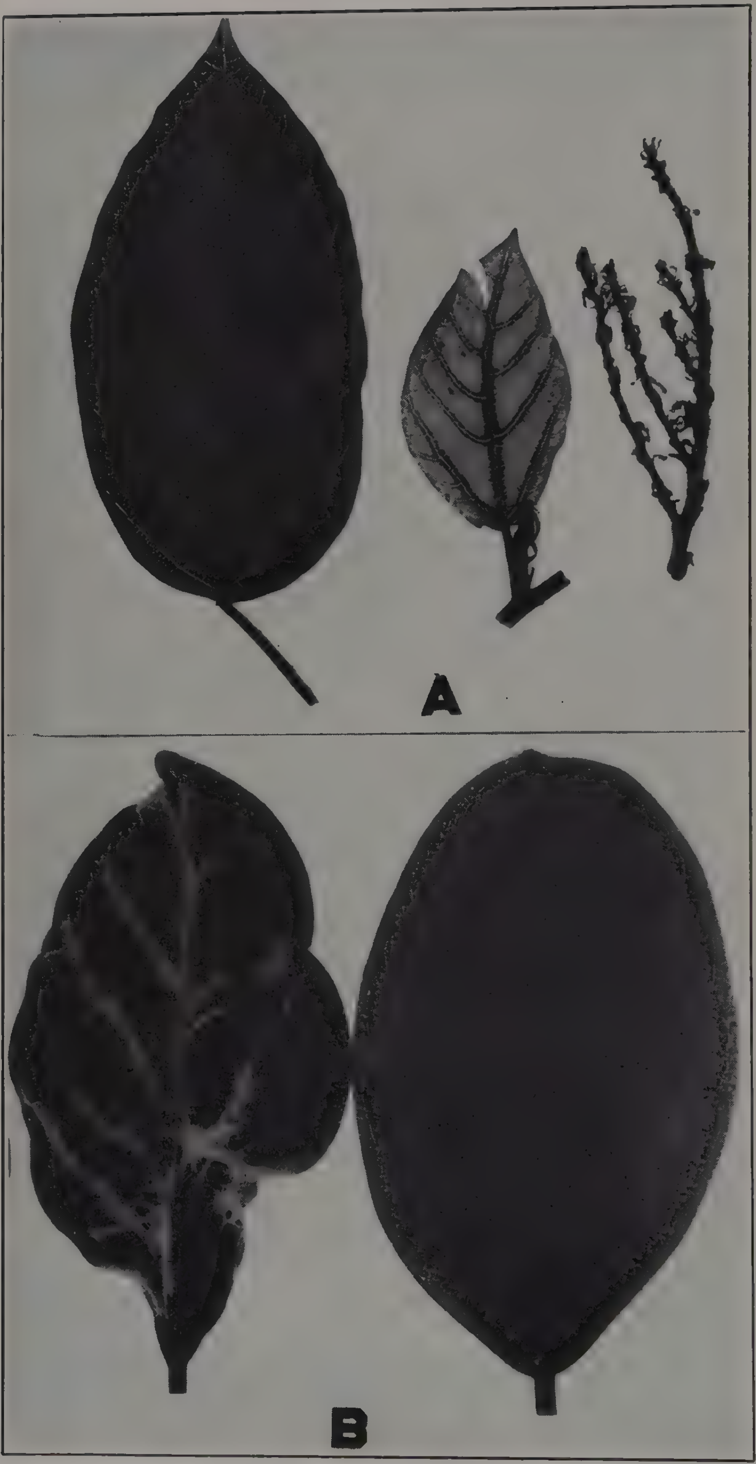

FIG. 2. A. Little leaf has appeared on *Ficus glomerata,* following severe frost injury to the tree; normal leaf at left. B. A mosaicked leaf, left, of *Ficus altissima,* contrasts strongly with a normal leaf, right.

reported in England, in Porto Rico, Canton and Nanking, China, New South Wales, Western Australia, and Texas. Pittman[2] found "fig leaf mottle" exceedingly common in Western Australia, where the disease "not only affects the leaves but frequently causes the first crop of figs to fall away without developing normally, and it may interfere also, at times, with the development of a considerable proportion of the second crop." Beneficial effects were reported by Pittman as a result of the application of copper sulphate to the soil under branches of mottled trees.

During the course of a recent trip through the southern and eastern United States, Condit found fig mosaic virtually nonexistent. It was seen on trees grown from cuttings recently obtained from California, but "fig bushes," long established in dooryards and in small commercial plantings, show foliage free from the characteristic mottling caused by mosaic.

Mosaic is apparently the cause of the peculiar spot or blemish of Samson caprifig in California, described and figured by Condit[3] in 1922. This mosaic spot, which brings about malformation and premature development of many Samson figs of the profichi or June crop, has also been found on many seedling figs at Riverside, but only on seedlings of the caprifig type.

The foliage of some fig seedlings shows abnormal coloration or spotting (Fig. 1). This appears to be more of the nature of a deficiency disease rather than of a mosaic. No remedial treatments have been attempted in either case.

A form of little leaf (Fig. 2, A) has recently been found on a single tree of *Ficus glomerata* growing at the Citrus Experiment Station, Riverside. This tree was frozen to the ground in January, 1937, when the temperature dropped to approximately 24° F. On some suckers the leaves are normal; on some the leaves are small, chlorotic, and malformed; on others the foliage is almost or entirely absent.

Leaf mosaic appears to be spreading from *Ficus carica* to other species. Leaves of *Ficus altissima* (Fig. 2, B) *F. krishna*, and *F. tsiela* growing in pots have all developed typical symptoms of mosaic with light patches of color in the blade and with some leaf malformations. The only insects known to have infested pot plants of the *Ficus* collection are red scale and a species of aphis. Ho and Li[4] found at Canton no convincing evidence of mosaic on fig trees other than *Ficus carica*, although some species showed evidence of its presence. No mosaic has been reported on foliage of *Ficus* in the collection at Chapman Field or elsewhere in Florida.

According to the recent suggestion by Fawcett (Science **92**: 559) for naming plant viruses, we propose the name **Ficivir caricae** for fig mosaic.—IRA J. CONDIT and W. T. HORNE, Citrus Experiment Station, Riverside, California.

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[2] Pittman, H. A. Fig leaf mottle. W. Australia Dept. Agr. Jour. 12: 196. 1935.

[3] Condit, Ira J. Caprifigs and caprifications. Calif. Agr. Exp. Stat. Bull. 319, pp. 356 and 370. 1922.

[4] Ho, W. T., and L. Y. Li. Preliminary notes on the virus diseases of some economic plants in Kwangtung Province, Lingnan Sci. Jour. 15: 69–70. 1936.

The Effect of Sodium Nitrate on Fusarium oxysporum cubense.—The statement was made by Wardlaw[1] that infection close to the root head of banana was the important factor in the Panama disease problem. The injury to the root usually was walled off before the fungus reached the head. Croucher[2] recommends that 2 oz. of sodium nitrate be applied to each root every 4 weeks. This fertilizer is applied close to the root head in the area of the greatest danger from Panama disease.

In order to determine the effect of the sodium nitrate on the causal organism, *Fusarium oxysporum cubense,* various dilutions of sodium nitrate were added to well-established soil cultures. When 16.6 parts of sodium nitrate were added to 83.4 parts of soil culture, the spores were inactivated, and only a few recovered sufficiently to germinate when removed to rice agar.

A study was made of the penetration of *Fusarium oxysporum cubense* in clay soil and sodium nitrate mixtures. After 16 days the penetration of the organism was measured. The results are shown in table 1. A mixture containing 1.538 per cent of sodium nitrate slowed down the growth of the organism. There was no penetration observed when 3.125 per cent was mixed with the soil.

TABLE 1.—*The penetration of Fusarium oxysporum cubense in mixtures of clay soil and sodium nitrate*

Tube No.	Soil	Sodium nitrate		Penetration in 16 days
	Grams	*Grams*	*Per cent*	*cm.*
1	0.0	40.0	100.0	None
2	20.0	20.0	50.0	....
3	30.0	10.0	25.0	....
4	35.0	5.0	12.5	....
5	37.5	2.5	6.25	....
6	38.75	1.25	3.125	....
7	40.0	0.625	1.538	2.7
8	40.0	0.312	0.773	3.8
9	40.0	0.156	0.388	4.8
10	40.0	0.078	0.194	4.9
11	40.0	0.039	0.097	3.9
12	40.0	0.019	0.047	3.8
13	40.0	0.009	0.022	4.0
14	40.0	0.004	0.009	4.0
15	40.0	0.0	0.0	4.1
16	40.0	0.0	0.0	3.4

Sodium nitrate is replacing other nitrogenous fertilizers on many of the larger properties in Jamaica.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Highgate, Jamaica, B. W. I.

Myriogenospora on Sugar Cane in Louisiana.—In July, 1940, the junior writer found in a commercial field of first stubble of the sugar-cane variety

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[1] Wardlaw, C. W. The biology of banana wilt (Panama disease) I. Root inoculation experiment. Ann. Bot. 44: 741–766. 1930.

[2] Croucher, H. H., and W. K. Mitchell. Fertilizer investigations with the Gros Michel banana. Dept. of Sci. and Agr. (Jamaica) Bull. 19. 1940.

C. P. 28/19, near Raceland, La., several plants that were characterized by extreme stunting and the adherence of the tips of the unfolding leaves to adjacent leaves that had arisen earlier. As the new leaf elongated, its tip was held down firmly along the midrib of the previously unfolded leaf by a black fungous stroma, giving the top of the plant a characteristic whip-like appearance (Fig. 1, A). In some instances the growing point was killed, followed occasionally by the death of the entire shoot, but more frequently by the development of new shoots from the lateral or basal buds. During the summer this successive death and sprouting of new shoots continued, with few of them attaining a height of more than 2 feet. Not all of the shoots died, some retaining a normal healthy color until fall, although they

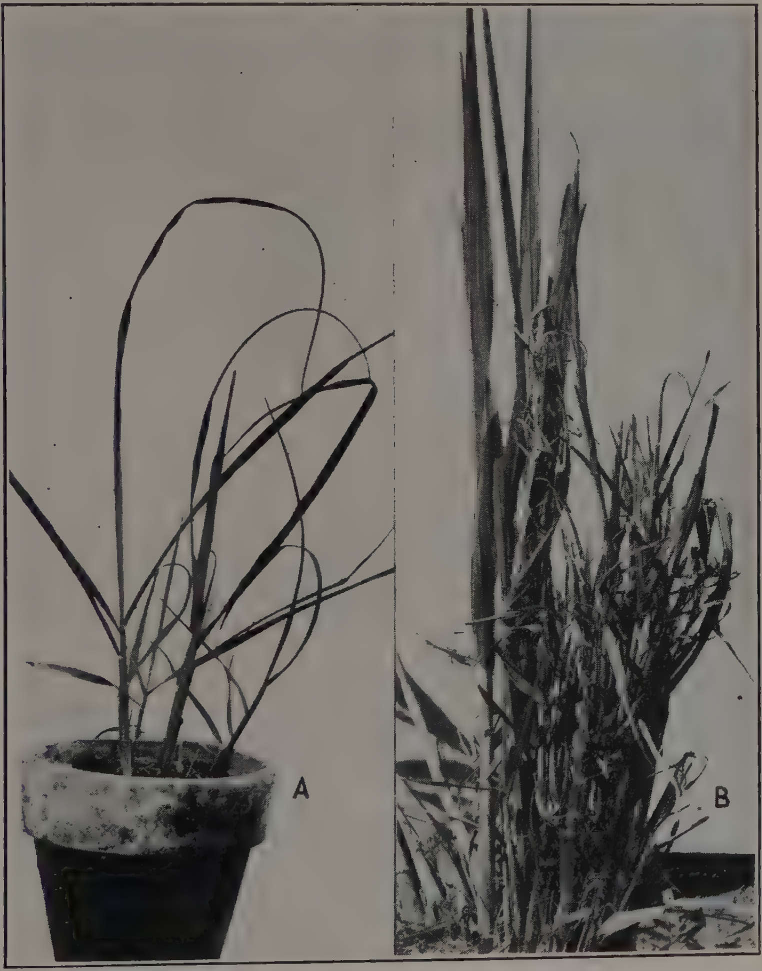

FIG. 1. *Myriogenospora aciculisporae* on sugar cane. A. Whip-like appearance of diseased shoots caused by adherence of tips of unfolding leaves to adjacent unfolded ones. Plant grown in greenhouse from naturally infected bud. B. Severely stunted diseased stool in the field.

remained severely stunted. In general, the symptoms agreed with those of the disease described by Vizioli,[1] as caused by *Myriogenospora aciculisporae.* A severely stunted diseased stool, photographed in the fall when healthy cane was 6 to 8 feet tall, is shown in figure 1, B.

Sections of diseased leaves from the spindle showed the presence of mature perithecia of a fungus in the upper portions, with progressively less mature stages occurring toward the growing point. Ascospore measurements and other characters of the fungus fell within the limits of Vizioli's description of *Myriogenospora aciculisporae.*

A total of 3 shoots in the 8 diseased stools developed into apparently healthy, mature stalks. However, they grew to only 3 or 4 feet in height and were small in diameter. These stalks were cut into single-bud pieces, which were potted in the greenhouse. All of them gave rise to apparently healthy plants. Of 3 lateral buds from a diseased stalk potted in the greenhouse, only 1 gave rise to a diseased plant.

This appears to be the first record of the occurrence of this sugar-cane disease in the United States, or in any country other than Brazil and Argentina.[2] Thus far it has been found in only the one locality in Louisiana, and affecting only 1 variety of cane. While no extensive surveys have been made to determine its distribution, it seems probable that if it were prevalent or widespread, it would have been found elsewhere during the fairly general surveys for chlorotic streak that have been made in recent years by the writers and others in the Louisiana sugar-cane district.

Wild grasses, including *Andropogon,* growing in the vicinity of the diseased sugar cane, were examined, but no *Myriogenospora* infection was found on them. Diehl[3] has reported a species of *Myriogenospora* on *Andropogon* in Louisiana, which he identified tentatively as *M. Paspali* Atk., but stated that the 3 described species of the genus could not be distinguished by their recorded ascus and spore dimensions. It is possible, therefore, that the fungus found on sugar cane by the writers is the same as that with which Diehl worked on *Andropogon.* Greenhouse infection experiments are in progress.—E. V. ABBOTT and R. L. TIPPETT, Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, Houma, La.

Preparation of Inoculum with a Mechanical Liquefier.[1]—An instrument, now frequently referred to as a blender,[2] but also suitably called a high-speed mixer, homogenizer, or liquefier, has been used with great effectiveness at the Southeastern Regional Vegetable Breeding Laboratory in making inoculations with tomato leaf-spot fungi. Since there are innumerable possibilities in the application of the technique to other diseases and other hosts, a brief reminder to plant pathologists at this time is deemed appropriate.

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[1] Vizioli, J. Estudo preliminar sobre um novo pyrenomyceto parasita da canna. Bol. Agr. São Paulo 27: 60–69. 1926.

[2] Stevenson, J. A., and R. D. Rands. An annotated list of the fungi and bacteria associated with sugar cane and its products. Hawaiian Planters' Record 42: 247–313. 1938.

[3] Diehl, W. W. A Myriogenospora disease of grasses. Phytopath. 24: 677–681. 1934.

[1] Contribution No. 10 from U. S. Vegetable Breeding Laboratory, Charleston, S. C.

[2] Waring Corp., 1697 Broadway, N. Y.; Modern Diet Products Supply Co., Milwaukee, Wisc.; Montgomery Ward & Co.

Effective mass inoculations with leaf-spot fungi are ordinarily dependent on a copious spore production; and, since the tomato early-blight organism (*Macrosporium solani* E. and M.) and the grey leaf-spot organism (*Stemphylium solani* Weber) do not produce conidia in culture in the great quantity desired for inoculation, it formerly had been necessary to resort to special techniques to stimulate spore formation.

The method involving use of the blender eliminates the need for conidia, or any other spore forms, and utilizes mycelial fragments that result from a brief processing of the fungus mats developed in liquid media. The mycelial fragments are quite as effective as conidia, and complete defoliation of greenhouse tomato plants has been obtained within 48 hours after inoculation with such material of *Macrosporium solani.*

Although the organisms used thus far have been grown on liquid media (Modified Leonian), cultures grown on semi-solid media, such as agar, bean pods, rice, potato cylinders, could be used equally well, since the machine is designed for the efficient maceration and liquefaction of raw fruits and vegetables. The machine used was purchased for preparing plant extracts for chemical determinations.

The slicing and beating of the blender are accompanied by strong centrifugal action. The instrument is efficient, and in 5 minutes or less will reduce coarse fungus mats to particles small enough to pass through a fine spray nozzle.

The procedure used to inoculate with tomato leaf-spot fungi is to dip the foliage of potted tomato plants in the liquid inoculum. However, the effectiveness of the liquified inoculum applied as a spray has been demonstrated in the case of *Stemphylium solani* and *Macrosporium solani* on tomatoes and of *Colletotrichum lindemuthianum* on beans.—C. F. ANDRUS, Bureau of Plant Industry, U. S. Dept. Agr., U. S. S. E. Regional Vegetable Breeding Laboratory, Charleston, S. C.

The Mycorhizal Fungi and Mycorhizae of Four Coniferous Plantations in the Rhine Valley.[1, 2]—A request by several foresters in Süd-Baden for further information concerning the ectotrophic mycorhizae of coniferous plantations in the Rhine Valley initiated a study on 4 coniferous plantations in the Black Forest near Karlsruhe to determine the responses of mycorhizal formation in sandy and humus soils and the effect of such growth upon the general health and probable yield from the stands. A survey of the hymenomycetes that appeared to support this phenomenon also was conducted.

[1] This study was made during the tenure of a Foreign Exchange Scholarship in the Forstliches Institut at A. Ludwigs Universität, Freiburg i/Br., Germany.

[2] The writer wishes to acknowledge the helpful suggestions by Dr. W. A. Kreutzer, Colorado State College, and the aid given by Dr. Fritz Sprau, Forst-pathologisches Institut at Munich, in the identification of the fungi.

Four 1-hectare plots were selected at random, each of which included plantations of young *Pinus silvestris, P. strobus, Picea abies,* and *Larix europaea.* Two of these plots had sandy soil, and 2 had a rich covering of humus.

In order to avoid two possible causes for low yield and the scarcity of mycorhizae, the nitrogen content and pH of the soils were tested.

Hyphal strands of the Hymenomycetes were traced from the fruiting bodies to the roots of saplings, and these roots were examined for the presence of ectotrophic mycorhizae.

In table 1 are given the results of tests made to determine the nitrogen content and the pH of the soils in the plots. Readings were recorded for the nitrogen content of the top soil and the subsoil at a depth of 6 inches.

TABLE 1.—*Nitrogen content and pH of the soils of the four plots studied*

Soil type	Plot	pH	Nitrogen content (p.p.m.)	
			Top soil	Sub-soil
Sandy	A	7.5	0.049	0.023
	B	6.8	0.039	0.021
Humus	C[a]	4.6	0.331	0.088
	D	4.3	0.344	0.096

[a] Formerly an oak plantation site.

The data given show that the pH values of the humus soils from plots C and D were relatively low, whereas those of the sandy soils from plots A and B were considerably higher. There was but a slight variation between the pH values of the soils in plots C and D.

It may be noted that plots A and B had relatively low nitrogen contents,

TABLE 2.—*Trees bearing ectotrophic mycorhizae and their corresponding mycorhizal fungi*

Mycorhizal hymenomycete	Trees bearing ectotrophic mycorhizae			
	Pinus silvestris	*Pinus strobus*	*Picea abies*	*Larix europaea*
Amanita muscaria	+	+	+	+
Boletinus porosus	−	+	−	−
Boletus badius	+	−	−	−
" *bovinus*	−	+	−	−
" *castaneae*	−	+	−	−
" *elegans*	−	−	−	+
" *granulatis*	+	+	−	−
" *lutens*	+	+	+	+
" *variegatus*	+	−	−	+
Cantharellus cibarius	−	+	−	−
Cortinarius balteatus	−	−	+	−
" *camphoratus*	−	−	−	+
" *mucosus*	−	−	−	−
Lactarius chrysorrheus	−	+	−	−
" *deliciosus*	+	+	+	−
Russula fragilis	+	−	−	−

whereas plots C and D had comparatively higher nitrogen contents. Differences in nitrogen contents betwen plots on the same soil type appeared to be of approximately the same degree.

Neither Hymenomycetes nor mycorhizae were observed on either sandy plot; they were scarce on one of the humus plots, which formerly had been the site of an oak plantation, while on the other humus plot Hymenomycetes and mycorhizae were numerous. In table 2 is given a list of Hymenomycetes that appeared to support the formation of mycorhizae, and the hosts that were observed to show such growth. As shown in this table, several of the fungi were not limited to one, but were found to support mycorhizal formation on several hosts.

Only the trees on plot D, which was rich in humus covering and on which numerous mycorhizae were found, appeared to be healthy and to give promise of a high yield. The trees on the 3 remaining plots generally showed signs of nitrogen starvation, and gave no indication of a good yield.—W. D. THOMAS, JR., Colorado State College, Fort Collins, Colorado.

Fluorescence of Diseased Potatoes.—The fact that cut surfaces of Irish potato tubers, affected with certain diseases, will fluoresce under ultraviolet radiation has been noted and discussed by a number of investigators, including Iverson and Kelly[1] and Harvey.[2] While it has been pointed out that tissues with various types of breakdown will fluoresce, the impression apparently prevails that the fluorescence is a reliable diagnostic symptom of ring rot (*Bacterium sepedonicum*).

During the present season it has been possible to examine diseased tubers from many cars of certified seed from various parts of the United States. In Louisiana there is no tolerance for ring rot in certified seed. If ring rot is found in a car the certification tags are removed and the potatoes cannot be sold as *certified seed.*

In the routine inspection work, most of the inspectors have been unable to distinguish satisfactorily between the ring rot and the so-called stem-end rot (apparently caused by *Fusarium eumartii*). Consequently, all potatoes found affected with either of these troubles have been sent to the laboratory for confirmation.

It has been observed that potatoes affected with the stem-end rot, as well as those affected with ring rot, fluoresce under ultra-violet radiation. This symptom, consequently, has been of little value in confirming the presence or absence of ring rot. In all cases it has been necessary to depend on the Gram stain.—L. H. FLINT and C. W. EDGERTON, Department of Botany, Louisiana State University, University, Louisiana.

[1] Iverson, V. E., and H. C. Kelly. A new method of identifying potato tubers free from bacterial ring rot and other types of tuber decay. Montana State College Agr. Exp. Stat. Mimeo. Circ. 20. 1940.

[2] Harvey, R. B. Florescence of potatoes under ultraviolet light for detecting ring rot. Phytopath. 31: 10. 1941.

The Use of Methyl Bromide as a Means of Detecting Latent Infections by Colletotrichum spp.[1]—In 1912, because the Mediterranean fruit fly (*Ceratitis capitata*) was found in Hawaii, Quarantine No. 2[2] was passed, prohibiting shipment of 30 vegetables and fruits to mainland United States. Later, the melon fly (*Dacus cucurbitae*) also was found in Hawaii, and, in 1914, Quarantine No. 2 was replaced by Quarantine No. 13.[3] The number of quarantined plant products was increased to 35.

In November, 1938, Hawaiian-grown fruits and vegetables were permitted entry into mainland States, provided the plant products had first undergone either a cold treatment or a vapor-heat treatment, designed to destroy the above-mentioned pests, and approved by the U. S. Bureau of Entomology and Plant Quarantine.[4] The use and value of these two treatments in Hawaii have been discussed fully elsewhere.[5,6]

Effective August 1, 1940, a third method of disinfestation[7] was approved for specific Hawaiian agricultural crops, including papayas and string beans. The method consists of fumigation with methyl bromide, 2 lb. per 1000 cu. ft., for 3½ hr. at atmospheric pressure and at a temperature not less than 80° F.

It has been found[8] that mature-green or firm-ripe papayas, treated with methyl bromide and held at 50° F. for 6 days (corresponding to shipboard refrigeration from Honolulu to California), must be kept at room temperature for several additional days in order to ripen. During this ripening period, anthracnose (*Colletotrichum* sp., probably *gloeosporioides* Penz.), develops rapidly on treated fruits, whereas nontreated fruits, otherwise similarly handled, exhibit little decay by the fungus. Fruits of both types are shown in figure 1, A. The *Colletotrichum* sp. becomes established in the still immature papaya fruits, as Wardlaw *et al.*[9] have previously shown and as local observations confirm, but remains latent until the fruits approach over maturity. Methyl bromide accentuates and accelerates the development of the fungus.

A somewhat similar phenomenon was observed when string beans (*Phaseolus vulgaris* var. lualualei) were treated with methyl bromide. Here the organism was *Colletotrichum lindemuthianum* (Sacc. and Magn.) Briosi

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[1] Published with the approval of the Director as Technical Paper No. 78 of the Hawaii Agricultural Experiment Station, Honolulu, T. H.

[2] Quarantine No. 2 (Domestic). Mediterranean Fruit Fly. September 18, 1912.

[3] Quarantine No. 13. Mediterranean Fruit Fly and Melon Fly. March 12, 1914.

[4] Strong, Lee A. Administrative instructions, the shipment of fruits and vegetables from Hawaii to the mainland subject to sterilization under supervision is authorized. B.E.P.Q. 481. 1938.

[5] Jones, W. W., J. J. Holzman, and A. G. Galloway. The effect of high-temperature sterilization on the Solo papaya. Hawaii Agr. Exp. Stat. Circ. 14. 1939.

[6] ————. Vapor-heat treatment for fruits and vegetables grown in Hawaii. Hawaii Agr. Exp. Stat. Circ. 16. 1940.

[7] Strong, Lee A. Administrative instructions amending authorization of the shipment of fruits and vegetables from Hawaii to the mainland subject to fumigation with methyl bromide under supervision. B.E.P.Q. 510. 1940.

[8] Jones, W. W. Methyl-bromide fumigation of papaya and tomato. Hawaii Agr. Exp. Stat. Circ. 17. 1940.

[9] Wardlaw, C. W., R. E. D. Baker, and S. H. Crowdy. Latent infections in tropical fruits. Trop. Agr. (Trinidad) 16: 275–276. 1939.

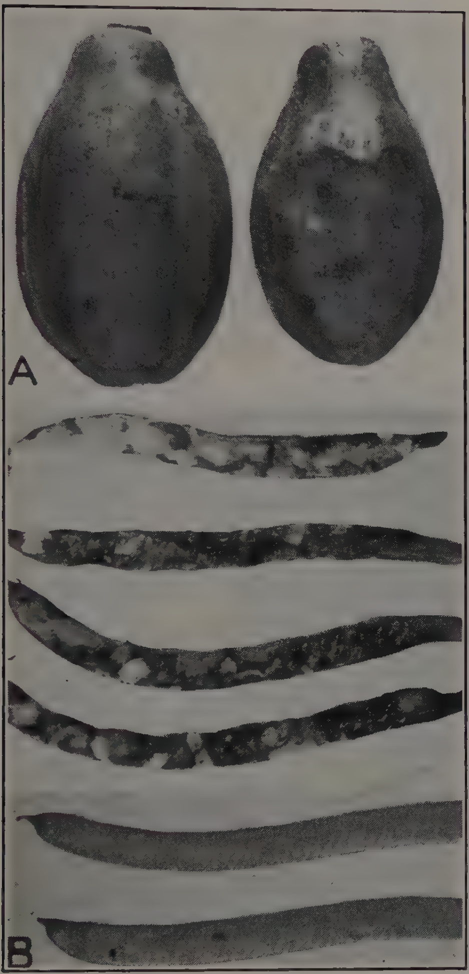

FIG. 1. Effect of methyl bromide in revealing latent infections by *Colletotrichum* spp. A. Untreated papaya fruit on left; fruit treated with methyl bromide on right. B. String beans treated with methyl bromide above; untreated, below.

and Cav. Treated pods were severely decayed, not only by the pathogen, but also by saprophytic species of *Fusarium* and *Alternaria*. These secondary fungi, in all cases, were growing on lesions that exhibited the characteristic acervuli and salmon-pink spore masses of *C. lindemuthianum*. Beans from the same lot, not exposed to methyl bromide, but otherwise similarly handled, remained free of fungus infection (Fig. 1, B).

The above findings are presented with the thought that methyl bromide, by its action in bringing to light infections that otherwise would remain temporarily or permanently latent, may be a useful agent in determining susceptibility to infection by fungi.—G. K. PARRIS and W. W. JONES, Hawaii Agricultural Experiment Station, Honolulu, T. H.

PRE–PUBLICATION NOTICE OF A REVIEW OF SEXUALITY IN THE HIGHER FUNGI BY PROFESSOR A. H. REGINALD BULLER

The *Botanical Review* will publish later this year a paper by Professor Buller covering his comprehensive studies and a review of contemporary literature in the field of cytology and physiology of reproduction in the higher fungi (Ascomycetes and Basidiomycetes). Perhaps a much larger number of workers in mycology and plant pathology than are regular subscribers to the *Review* may want personal copies of this article, and students in undergraduate and graduate courses in mycology will likely find this paper so essential an aid to the comprehension of this critical field of study that it will become one of their best-thumbed reference works. The article will, in fact, summarize much of the material that has been published during the last 25 years in the author's Researches on Fungi, of which the 6th volume appeared in 1934, but the 7th, though complete in manuscript, has been indefinitely postponed because of the war. It is not widely known that the entire unsold stocks of these books were destroyed in the Fire of London, on December 29, 1940, nor is it generally realized that very few sets exist in personal or, even institutional, libraries in the United States. Furthermore, since these books were Professor Buller's almost exclusive channel of publication, their unavailability may be painfully felt by future students of mycological literature.

As a service to plant pathologists generally, and with the concurrence of the editors of PHYTOPATHOLOGY, I deem it appropriate to call attention to the prospective publication of this review. I am informed that the author contemplates the purchase of only a limited number of separates, and that the cost of these is such that no extensive gratis distribution is feasible. However, at the instigation of his associates, Professor Buller has agreed to accept advance orders for separates, which will cover two whole numbers of the *Botanical Review,* at a cost of 50 cents. Remittance should be inclosed with order. To obviate difficulties in the exchange of currency between the United States and Canada, orders originating here should be sent to the Botanical Review (Fordham P. O., New York, N. Y.); those from Canada, to Professor Buller at the University of Manitoba.—FREEMAN WEISS.

SYMPOSIUM PAPERS AVAILABLE

The papers presented at the symposium on "Plant Quarantines as Barriers to Interstate Trade" under the auspices of the Section of Plant Quarantine of the American Association of Economic Entomologists, at Philadelphia in December 1940, have been mimeographed for the benefit of those interested. S. A. Rohwer, A. B. Buchholz, D. B. Mackie, Lee McClain, R. P. White, T. J. Headlee, and E. L. Chambers are the authors. As long as they last, copies may be obtained free by members of the American Phytopathological Society who address a request to: Howard P. Barss, Office of Experiment Stations, U. S. Department of Agriculture, Washington, D. C.

SUMMER MEETING OF THE NORTH CENTRAL STATES GROUP OF PHYTOPATHOLOGISTS

The North Central States group of phytopathologists will conduct a summer tour in southern Michigan from July 10 to 12. The group will assemble at St. Joseph, Michigan, on July 9. July 10 will be spent in the intensive fruit and vegetable area in Berrien and Van Buren Counties. July 11 will be devoted to visiting fruit, mint, celery, vegetable, cereal, and muck crops between South Haven and Kalamazoo. The night will be spent at East Lansing. On July 12 the group will visit experiments and laboratories at the college.

Members of the Society other than those in the North Central States (Michigan, Wisconsin, Minnesota, Iowa and Ohio) who plan to attend the meeting should write to Donald Cation for detailed program about June 10.

THE BEHAVIOR OF CERTAIN VIRUSES IN PLANT ROOTS[1]

ROBERT W. FULTON

(Accepted for publication November 18, 1940)

A characteristic feature of many viroses is the spread of the causative agent into all parts of the plant, with the exception, in certain instances, of the seeds. Many viroses have been regarded as completely systemic, however, without proof that the virus invades the roots. While there is apparently no reason to suppose that root invasion is not a regular occurrence, the presence and behavior of viruses in the roots has received less attention than the subject merits.

In connection with the physiology of viroses, it is of interest to know the extent to which virus behavior is modified in different organs of the plant. Since the physiologic functions of the roots are different from those of the leaves, viruses are subject to a set of conditions in the roots that differ correspondingly from those in the leaves and stems. It seemed likely that these differences might be of such nature and magnitude as to affect the behavior of viruses and that consideration of the virus in the roots would provide a better understanding of the host-virus relationship.

From a practical viewpoint, the question of root infection is still unsettled. Scattered reports in the literature indicate that direct infection of roots is not regularly secured by artificial inoculation, although viruses do appear to invade the roots readily following leaf inoculation. This apparent inconsistency has been explained in this investigation by the demonstration that, although roots are easily infected directly, the virus moves upward very slowly, and does not, within relatively long periods of time, enter the stem or induce leaf symptoms.

Since it was necessary to limit most of the work to only a few of the large number of viruses and plant species available for such studies, general conclusions may not be warranted. The data presented are sufficient to show, however, some marked differences in the behavior of viruses in the roots of tomato and tobacco as compared with the behavior in the leaves. It is believed that a knowledge of such differences is essential to a better understanding of virus diseases in plants.

REVIEW OF LITERATURE

The presence of viruses in plant roots, while not investigated extensively, has been reported in certain instances. The ordinary tobacco-mosaic virus was recovered, for example, from the roots of tobacco, tomato, and petunia by Allard (1, 2) as early as 1916. More recently, Price (28) has reported finding the tobacco-ringspot virus, and Smith (33) the tobacco-necrosis virus, in the roots of tobacco plants. The sugar beet curly-top virus, the tomato bunchy-top virus, and several others have been demonstrated in roots.

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[1] This investigation was conducted under allotments from the University of Wisconsin Research Fund. This paper was presented to the Graduate School of the University of Wisconsin in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

The writer is indebted to Dr. James Johnson for advice and criticism during the work and preparation of the manuscript.

Apparently, in only two instances was failure to demonstrate viruses in the roots of infected plants reported. Doolittle (13) did not recover ordinary cucumber-mosaic virus from the roots of infected cucumber plants, and Fajardo (17), using the root extract of mosaic bean plants, obtained no infection.

Conflicting reports have been published on the susceptibility of roots to direct infection. Allard (1, 3) concluded that wounded tobacco roots might be infected by the ordinary tobacco-mosaic virus, although infection did not occur regularly. Mulvania (27), Lehman (24) and Johnson (21) reported that attempted inoculations of tobacco roots with the ordinary tobacco-mosaic virus failed to cause leaf symptoms. White (37) could not infect sterile excised tomato roots *in vitro* by inoculating them directly with the ordinary tobacco-mosaic virus. According to Doolittle (13), cucumber-mosaic virus did not infect cucumber plants through the roots. Webb (36), however, obtained convincing evidence that the virus of wheat mosaic does regularly infect through the roots of wheat plants. Fajardo (17) obtained infection by immersing the cut ends of bean roots in an extract of bean-mosaic virus. By this same method, however, Doolittle and Wellman (14) were unable to infect celery plants with the celery-mosaic virus. One virus, on the other hand—that of phony disease of peach—has been transmitted only by root grafts (19).

The occurrence of a virus normally infesting only the roots of tobacco was reported by Smith (33). When inoculated to the leaves, the virus remained localized. Price (30), in comparing this virus with ordinary tobacco-mosaic virus, found that tobacco roots could be directly infected by each virus, but that leaf symptoms did not result from root infection. Bennett (9) reported that tobacco-mosaic virus could directly infect tobacco roots, but that upward movement of the virus into the leaves occurred only after relatively long periods, or after removal of the tops of the plants.

Studies on the movement of viruses in their host plants have been concerned mainly with the movement that occurs in the aerial parts of the plant. The literature on this subject is voluminous and need not be cited in detail here, particularly, since Esau (16), Crafts (11), and Bawden (5) have recently presented reviews. In brief, the experimental results have indicated that mechanically transmitted viruses move relatively slowly through the parenchyma until they reach vascular tissue. Upon invading the vascular tissue, the viruses move upward and downward in the stem with relative rapidity. The evidence has indicated that the phloem is the channel of this rapid systemic movement of viruses. In the case of the sugar-beet curly-top virus, particularly, Bennett (6, 8) has shown that the direction of movement is determined by factors which presumably determine the direction of movement of food materials in the plant. The evidence also indicates that the mechanism responsible for the movement of this virus is of the nature of a mass flow of solution in the phloem.

In addition to the problems of root invasion, direct infection, and movement of viruses in roots, questions have arisen as to the effect of the root tissues on the virus itself. It has been reported in several instances that viruses appear to be lower in concentration in the roots of infected plants than in the leaves. This was demonstrated by Price (28), using tobacco plants infected with the tobacco-ringspot virus. Matsumoto and Samazawa (26) found that the concentration of the antigen of tobacco-mosaic virus, presumably representing actual virus concentration, was approximately equal in fresh extracts of diseased tobacco leaves and roots. Stanley (35), however, ultracentrifugally extracting the protein of aucuba-mosaic virus, found that the yield of this protein (mg. per cc. of crude juice) from roots of infected tomato plants was only about one-fourth the yield from leaves.

That the ordinary tobacco-mosaic virus multiplies in root tissues was shown by White (37) by means of sterile roots excised from infected tomato plants and grown *in vitro*. He found that rate of multiplication of the virus in these roots seemed to be correlated with rate of root growth.

MATERIALS AND METHODS

The viruses employed in this investigation were those of ordinary tobacco mosaic (*tobacco virus 1*), ordinary cucumber mosaic (*cucumber virus 1*), severe etch, tobacco ring spot, and potato ring spot. In a few trials, the viruses of common bean mosaic, tobacco streak, and tobacco broad ring spot (22) were used.

A considerable number of genera and species of host plants were inoculated in certain preliminary phases of the investigation. The two host plants selected as particularly suitable for more detailed study were tobacco (*Nicotiana tabacum* var. Havana-seed) and tomato (*Lycopersicum esculentum* var. John Baer).

The plants were grown in composted soil in 4-in. pots. Readily available roots were obtained by inducing them to grow through the perforation in the bottom of the pot into water (Fig. 1). For this purpose, tall crocks were used that were wide enough to allow a pot to be inserted up to its rim. The space in the crock below the bottom of the pot was kept partly filled with water, and the soil was periodically watered from below by raising the water level in the crock above the base of the pot. The roots that grew out through the perforation were soil-free, and could be removed and tested for virus without appreciably disturbing the plant. Such roots also could be inoculated without introducing the virus into the soil, and virtually eliminated the possibility of the plants' becoming infected by contact of the stem or leaves with the virus.

During the time the roots were growing from the base of the pots, the plants were kept in a greenhouse at 19° to 24° C., this temperature being most favorable for root growth. Just before being inoculated, the plants were moved into a greenhouse kept at 27° to 32° C., a temperature more favorable for the progress of most virus diseases.

Leaves were inoculated in the usual manner by lightly rubbing or wiping them with a cheesecloth pad dipped in fresh virus extract. The leaves to be inoculated, except those of tobacco and tomato, were first dusted with a small amount of carborundum powder. Root inoculations were made also with carborundum powder, which was added to the infectious extract before the roots were rubbed. All plants were relatively young and actively growing when inoculated.

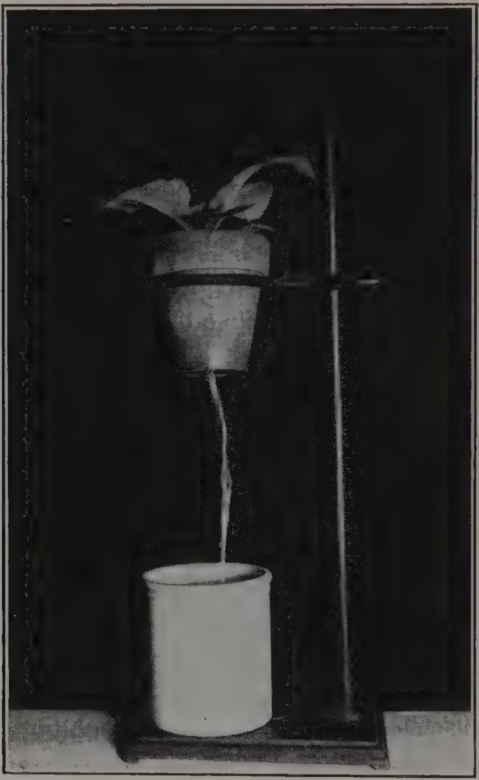

FIG. 1. Method of growing exposed roots for experimental purposes. The 4-inch pot is set directly in the 4-inch crock containing water which permits sub-irrigation when desired and forms a dark, moist chamber into which the roots grow through the perforation in the base of the pot.

The presence of virus in the tissue extracts was determined by wiping the extract on the leaves of 5 young healthy test plants. Since all but one of the viruses produced characteristic symptoms on tobacco, this plant was used almost exclusively for determining the infectivity of the various extracts. Seedling bean plants (*Phaseolus vulgaris* L. var. Common Refugee) were used to test for the presence of common bean-mosaic virus. In tests with ordinary tobacco-mosaic virus, the local lesions produced on leaves of the *Nicotiana glutinosa* × *N. tabacum* hybrid (20) were used to determine the presence of the virus. This plant is referred to simply as "the hybrid" in the text of this paper.

EXPERIMENTAL RESULTS

Root Invasion by Different Viruses

Since little is known of the regularity with which some viruses invade roots from aerial plant parts, further information on this subject was sought at the outset. Plants known to be susceptible to systemic invasion by one or more viruses were selected from a number of different families and genera. Four plants of the species to be tested were used in each trial with each virus. The roots of these plants were allowed to grow from the bottoms of the pots into crocks containing water. Several young leaves were inoculated with fresh leaf extract of the virus. At intervals of 5, 10, 15, and 20 days after inoculation, roots were removed from one of the 4 plants and tested for the presence of the virus originally introduced into the leaves.

Since the roots were tested for virus only every 5 days and only a few trials were run with each virus in a single host, data on the rate of invasion of the roots by the various viruses are probably not significant and are not included here. In over one-half of the trials, however, the virus appeared in the roots within 5 days after inoculation, while in 85 per cent of the tests, virus was present in the roots within 10 days after inoculation. It is evident, therefore, that root invasion by some viruses occurs relatively soon after infection of the leaves.

The following host plants were inoculated, species being grouped under the viruses found to invade the roots within 20 days after the leaves were inoculated:

ORDINARY TOBACCO-MOSAIC VIRUS

Cynoglossum amabile Stapf. and Drum.
Fagopyrum esculentum Gaertn.
Lycopersicum esculentum Mill.
Nicandra physaloides (L.) Pers.
Nicotiana tabacum L.
Petunia violacea Lindl.
Solanum nigrum L.
Spinacia oleracea L.

ORDINARY CUCUMBER-MOSAIC VIRUS

Apium graveolens L.
Capsicum fructescens L.
Cynoglossum amabile Stapf. and Drum.
Cucumis sativus L.
Fagopyrum esculentum Gaertn.
Hibiscus esculentus L.
Lycopersicum esculentum Mill.
Nicotiana glutinosa L.
N. tabacum L.
Solanum melongena L.
S. nigrum L.
Spinacia oleracea L.
Zinnia elegans Jacq.

POTATO-RING-SPOT VIRUS

Lycopersicum esculentum Mill.
Nicotiana glutinosa L.
N. tabacum L.
Solanum melongena L.
S. nigrum L.

BEAN-MOSAIC VIRUS

Phaseolus vulgaris L.

TOBACCO-RING-SPOT VIRUS

Amaranthus caudatus L.
Aster laevis L.
Calendula officinalis L.
Cucumis sativus L.
Cynoglossum amabile Stapf. and Drum.
Datura stramonium L.
Dolichos lablab. L.
Helianthus annuus L.
Hibiscus esculentus L.
Nicandra physaloides (L.) Pers.
Nicotiana glutinosa L.
N. tabacum L.
Petunia violacea Lindl.
Salvia splendens Ker.
Solanum melongena L.
Spinacia oleracea Lindl.
Zinnia elegans Jacq.

SEVERE-ETCH VIRUS

Datura stramonium L.
Lycopersicum esculentum Mill.
Nicandra physaloides (L.) Pers.
Nicotiana glutinosa L.
N. tabacum L.
Petunia violacea Lindl.
Solanum nigrum L.

TOBACCO-STREAK VIRUS

Datura stramonium L.
Nicotiana glutinosa L.
N. tabacum L.

BROAD-RING-SPOT VIRUS

Datura stramonium L.
Nicotiana tabacum L.

In the roots of a few species, no virus was found within 20 days after inoculation. The young, noninoculated leaves of these plants, when tested as inoculum, also failed to cause infection. It seems safe to assume, therefore, that in every case where the virus became systemic in the leaves and stems it also entered the roots. In all, 8 different viruses were recovered from the roots of a total of 56 species of plants.

Susceptibility of Roots to Direct Infection

Although systemic invasion of roots by different viruses inoculated to the leaves occurs readily, previous reports are conflicting on the question of whether or not roots can be infected by sap inoculation. As has been mentioned, Price (30) and Bennett (9) have recently shown that tobacco roots may be directly infected with ordinary tobacco-mosaic virus, the virus remaining more or less confined to the roots. In view of earlier reports by others of failure to infect plants through the roots, it seemed worth-while to attempt to obtain additional information on this point and to extend the observations to viruses other than the ordinary tobacco-mosaic virus.

The recovery of the ordinary tobacco-mosaic virus from inoculated roots may not be conclusive evidence of infection because of the possible persistence on the root surfaces of the virus originally applied as inoculum. It was believed that if viruses ordinarily inactivated in a few days by aging could be recovered several weeks after roots were inoculated, this would offer better evidence that root infection had occurred. By this method evidence of the direct infection of tobacco roots by the viruses of tobacco-ring-spot and ordinary cucumber-mosaic was obtained. These viruses were recovered from tobacco roots 28 days after the roots were inoculated. In each case 10 out of 10 young tobacco plants were infected by the extract of the inoculated roots. No symptoms developed on the leaves of the root-inoculated plants, and virus could not be recovered from the leaves.

Another criterion was used to determine whether tobacco roots could be infected directly by ordinary tobacco-mosaic virus. The presence of inclusion bodies in the cells of inoculated roots should offer direct proof that infection has been obtained. Accordingly, the roots of several young tobacco plants were rubbed with fresh extract of ordinary tobacco-mosaic virus and immediately washed in running water. Fourteen days later, pieces of the roots were cut from the plants and fixed in formalin acetic-acid alcohol fixative. As controls, noninoculated roots from healthy plants and roots from typically mosaic plants were also fixed. Paraffin sections of these roots were stained by the iron alum-haematoxylin method.

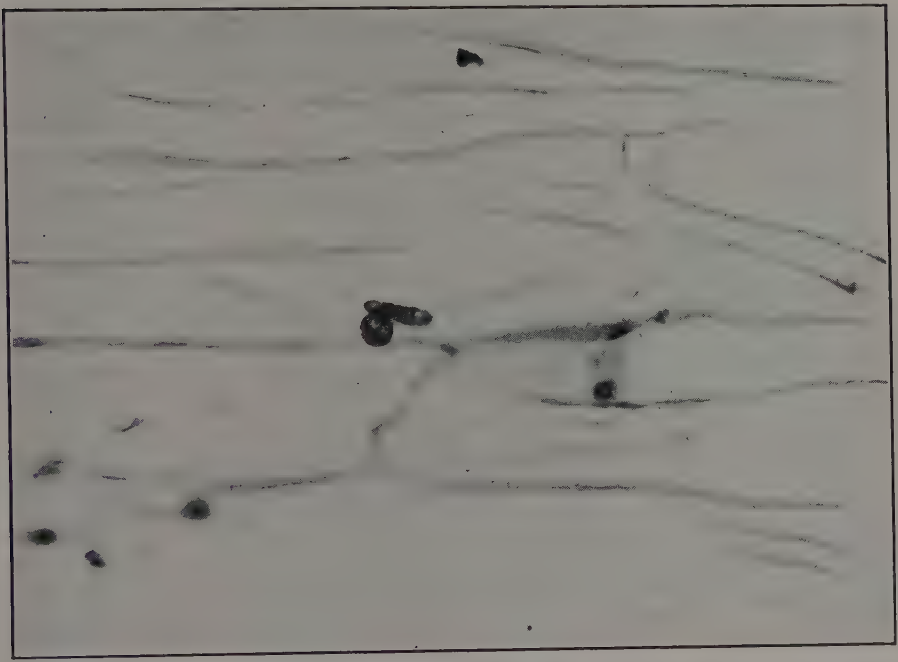

FIG. 2. Photomicrograph of longitudinal section of tobacco root stained with iron-alum haematoxylin. The root had been inoculated with tobacco mosaic virus 14 days previously. An x-body lies beside the nucleus.

Inclusion bodies, both x-bodies (Fig. 2) and striate material, were found abundantly in directly inoculated roots. These bodies did not differ from those found in roots infected by invasion of the virus from the leaves. No inclusion bodies were found in the noninoculated control roots. The plants from which directly inoculated roots had been removed were held for an additional 30 days. At the end of this time, no symptoms had developed and no virus could be recovered from the leaves. Similar inclusion bodies also were found in tomato roots similarly inoculated. These results confirm statements that roots may be infected by direct inoculation with the ordinary tobacco-mosaic virus. The infection, however, is evidently confined to the roots, as the aerial parts of the plants remain apparently healthy.

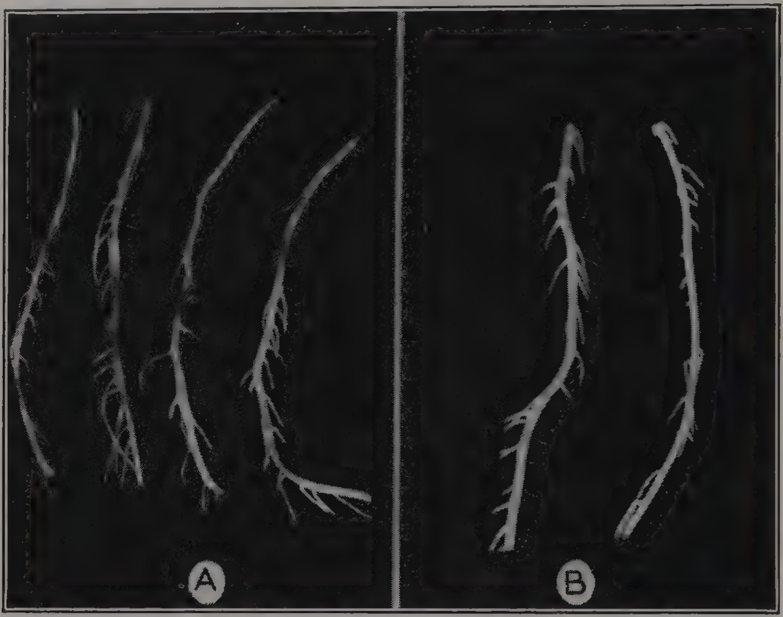

FIG. 3. Local lesions on roots of hybrid (*Nicotiana tabacum* × *N. glutinosa*) resulting from direct inoculation with ordinary tobacco mosaic virus. A. Roots inoculated by wiping with infectious extract. B. Roots wiped with extract from healthy plants.

Root infections differed from leaf infections in that no macroscopic symptoms developed on the roots as a result of direct infection. Neither have symptoms been observed on any roots of plants systemically invaded from aerial parts by the viruses used in this investigation. A necrotic type of symptom may develop on roots, however, as was shown by rubbing the roots of the hybrid (*Nicotiana tabacum* × *N. glutinosa*) with the extract of ordinary tobacco-mosaic virus. Four to 5 days after inoculation, elongate, light-brown spots appeared on the older roots (Fig. 3). Lesions completely encircled the smaller rootlets and caused them to break when slightly disturbed. Within 8 to 10 days after inoculation, the entire root system was brown and soft, perhaps partly as a result of secondary invasion by microorganisms. Portions of the roots that remained under water after inoculation showed soft, indefinite lesions with little discoloration. Hybrid roots, rubbed with healthy tobacco extract containing carborundum powder, remained healthy in appearance. Using other methods of inoculation with the ordinary tobacco-mosaic virus, Johnson (21) had previously failed to secure symptoms on the roots of the hybrid host.

Movement of Viruses in Inoculated Roots

The observation that direct infection of the roots did not result in leaf symptoms suggested that the movement of the virus was greatly restricted in the roots. In order to determine the rate and direction of virus movement subsequent to root infection of tomato and tobacco, the following method was used. The individual roots of each plant in a series of 4 or 5 plants were separated and straightened so that loops did not occur. A 1-in. section of the roots about 3 to 4 inches below the bottom of the pot was marked for inoculation by tying 2 threads lightly around the roots (Fig. 4). The roots between the threads were inoculated by rubbing with infectious extract containing carborundum powder, taking precautions to avoid contaminating the remainder of the root system with the inoculum. The roots were then washed for about 30 seconds in running water.

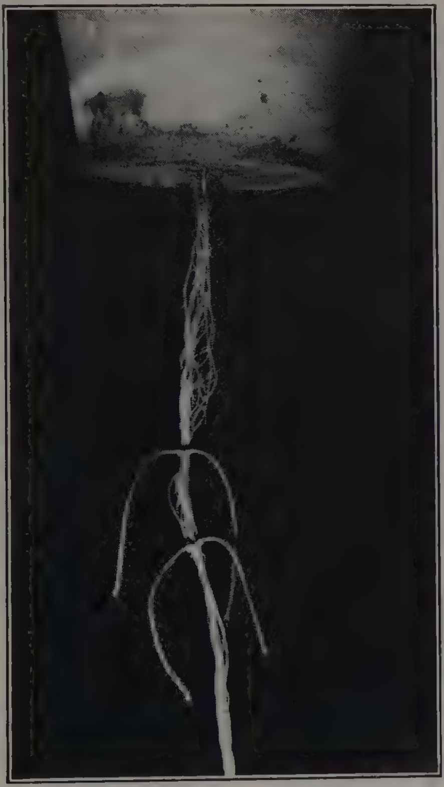

FIG. 4. The rate and direction of virus movement in roots was investigated by means of inoculating marked sections of roots as shown here and returning the roots to the moist chamber where they were grown.

At daily intervals after inoculation, the roots were cut into 1-in. sections at definite distances above and below the inoculated sections. These pieces of root tissue were then macerated with a few drops of water and inoculated to either the hybrid host or 5 young Havana-seed tobacco plants.

Results showing the rate and direction of movement of ordinary tobacco-mosaic virus in inoculated roots of tobacco and tomato are presented in table 1. It is apparent that downward movement in the roots occurred rapidly

TABLE 1.—*Rate of movement of tobacco-mosaic virus upward and downward from the point of inoculation of the roots*

Host	Position of root sections tested, inches above or below inoculated section	Average number of local lesions on hybrid leaves from three trials with root sections taken at positions and intervals (days) shown after inoculation				
		2	3	4	5	6
Tobacco	2–3 above	0	0	0	0	0
	1–2 do	0	0	0	0	17
	0–1 do	0	44	57	44	61
	Inoculated section	35	367	404	428	328
	0–1 below	0	6	43	109	301
	1–2 do	0	1	38	79	400[a]
	2–3 do	0	3	48	61	413
	Over 3 do	0	8	56	42	327
Tomato	2–3 above	0	0	0	0	
	1–2 do	0	0	0	0	
	0–1 do	0	6	60[a]	7	
	Inoculated section	34	136	250[a]	172	
	0–1 below	0	0	80	200[a]	
	1–2 do	0	10	62	300[a]	
	2–3 do	0	6	133	450[a]	
	Over 3 do	0	17	150[a]	450[a]	

[a] Estimated.

but that upward movement was very slow. In both hosts, the virus remained localized in the inoculated area for 2 to 3 days, then descended 4 inches within one 24-hour period.

The results of similar trials with several other viruses in tobacco and tomato roots are presented in table 2. All the viruses used in both hosts moved upward in the roots only very slowly. The downward movement, on the other hand, usually occured rapidly. The viruses differed in the length of time they remained localized in the inoculated section. Cucumber-mosaic virus in tobacco roots, for example, remained localized in the inoculated area for 4 days, while tobacco-mosaic virus remained localized only 2 days.

The results with potato-ring-spot virus in tobacco roots are not tabulated because, with few exceptions, the virus was recovered only from the inoculated section in the 10-day period the trials were run. The exceptions were recovery of virus in a few instances from sections immediately above or below the one inoculated. The results with tobacco-ring-spot virus are somewhat similar, except that in two instances the virus more or less completely invaded the roots below the point of inoculation. It is perhaps significant that the upward movement of these two ring-spot viruses in tobacco roots was also much slower than for the other viruses tested.

Samuel (32) pointed out that if viruses are transported in the phloem, then all viruses in the same host should move at the same rate, but might differ in the time required to move through parenchymatous cells and enter the phloem. This is apparently the case with the downward movement of different viruses in inoculated tomato and tobacco roots. Presumably the two viruses that remained localized, or nearly so, moved so slowly through

TABLE 2.—*Rates of movement of different viruses upward and downward from the point of inoculation of the roots of tobacco and tomato*

Virus and host	Position of root sections tested, inches above or below inoculated section	Number of times[a] virus was recovered in three trials from root sections taken at positions shown at following daily intervals				
		2 days	3 days	4 days	5 days	6 days
Severe-etch virus in tomato	1–2 above	0	0	0	0	
	0–1 do	0	1	1	2	
	Inoculated section	2	2	3	3	
	1–2 below	0	1	3	3	
	Over 3 do	0	1	3	3	
Potato-ring-spot virus in tomato	1–2 above	0	0	0	0	
	0–1 do	1	3	1	2	
	Inoculated section	3	3	3	3	
	1–2 below	0	0	3	3	
	Over 3 do	0	0	3	3	
Cucumber-mosaic virus in tobacco	1–2 above		0	0	0	0
	0–1 do		0	1	0	1
	Inoculated section		2	3	3	3
	1–2 below		0	0	2	3
	Over 3 do		0	0	1	3
Severe-etch virus in tobacco	2–3 above	0	0	0	0	
	1–2 do	0	0	0	1	
	0–1 do	0	1	1	2	
	Inoculated section	2	2	3	3	
	1–2 below	0	1	3	2	
	Over 3 do	0	1	3	3	
		7 days	8 days	9 days	10 days	11 days
Tobacco-ring-spot virus in tobacco	1–2 above	0	0	0	0	0
	0–1 do	0	1	0	0	0
	Inoculated section	2	3	3	3	3
	1–2 below	0	0	2	0	0
	Over 3 do	0	0	1	0	0

[a] Five tobacco plants were used in each trial. One or more infected plants were recorded as positive infection for the trial.

the parenchyma that they did not reach the phloem within the periods of the experiments. It will be noticed that, in general, the viruses that remained localized the shortest time were those that ultimately moved farthest upward. It seems likely therefore, that the period of localization and the upward movement in the roots are dependent upon the relatively slow movement of the virus through parenchyma tissue.

Virus Movement in Leaves and Stems as Compared with Movement in Roots

The type of virus movement observed in roots differs in some respects from that observed in leaves and stems by other workers. In the roots, viruses move rapidly only downward, but it often has been reported (9, 18, 23, 32) that, in stems, viruses move relatively rapidly both upward and downward. Previous work on virus movement in tobacco and tomato, however, has been confined to one or two viruses, and the experiments conducted under conditions that may have differed somewhat from those used in the present investigation. For this reason trials were made to determine the rate of movement of several different viruses following leaf inoculation of tomato and tobacco, with the purpose of comparing the results with those of tests on virus movement in the roots.

The plants used varied in different tests from 6 to 12 in. in height (6 to 14 leaves), but were of a uniform size for each trial. The roots of these plants were allowed to grow into the crocks as in the previous experiments. The single leaf inoculated in all cases was the third from the top of the plant, the first leaf counted being about 1 inch long in each case.

Data on the movement of the virus were obtained in two ways. At daily intervals after inoculation, the inoculated leaf was removed from one plant. The subsequent presence or absence of symptoms indicated whether or not the virus had moved from the inoculated leaf into the stem within the time interval allowed. At the same time that the inoculated leaf was removed the roots were also removed and tested for the presence of virus by inoculation to healthy plants. This method provided a measure of the time a virus remained localized in the inoculated leaf and the total time required for downward invasion of the roots (Table 3).

TABLE 3.—*Comparison of rates of downward movement of different viruses from a single inoculated leaf of tobacco and tomato plants*

Host	Virus of	Number of trials	Average time (hours) between inoculation and first appearance of virus in stem	Average time (hours) between inoculation and first appearance of virus in roots	Average difference in hours between first appearance of virus in stem and first appearance in roots
Tobacco	Ordinary tobacco mosaic	11	60	81	21
do	Ordinary cucumber mosaic	5	84	104	20
do[a]	Potato ring-spot	3	....	240[c]	......
do[b]	Potato ring-spot	3	168	224	56
do	Tobacco streak	3	88	112	24
do	Severe etch	4	48	72	24
do	Tobacco ring-spot	4	136	210	74
Tomato	Ordinary tobacco mosaic	5	64	84	20
do	Ordinary cucumber mosaic	3	184	200	16
do	Potato ring-spot	5	53	77	24
do	Severe etch	5	62	91	29

[a] Plants 6 inches or more in height.
[b] Plants 4 inches or less in height.
[c] No virus in the roots in the time indicated, when the trials were terminated.

The viruses differed from one another in the length of time they remained confined to the inoculated leaf. This period largely determined the total time required for the viruses to reach the roots, since, with two exceptions (tobacco-ring-spot and potato-ring-spot viruses in tobacco plants), each virus appeared in the roots in about 24 hours after it first appeared in the stem. That this period does not represent the time used by the virus in moving down the stem is shown by the observation that a virus entered the roots of plants 12 in. tall quite as soon as the roots of plants half as tall. A possible explanation is that small amounts of virus present in the stems in the early stages of invasion multiplied and eventually induced symptoms, but that the same amount of virus was not detected in the roots when they were used as inoculum. It seems evident that the viruses used, except that of potato-ring-spot in tobacco, moved downward into the roots at fairly rapid rates. These results are in general accord with those of other investigators with respect to the rate of movement in stems.

The movement of potato-ring-spot virus and tobacco-ring-spot virus in tobacco differs somewhat from that of the other viruses. Only when tobacco plants 4 in. high or less were inoculated did the potato-ring-spot virus move into the roots and then not consistently. The movement of tobacco-ring-spot virus in tobacco was somewhat similar, although systemic movement did occur in fairly large plants. With both these ring-spot viruses in tobacco, 2 or 3 days elapsed between the first appearance of the virus in the stem and roots, respectively. These viruses either were not detected in the roots for 1 or 2 days after root invasion occurred, or they moved more slowly down the stem and into the roots than other viruses used in these trials.

A comparison of the relative time required for downward movement of several viruses in the roots with the time required for downward movement in the leaves and stems is presented in table 4. These figures show that the time required for the viruses to enter the systemic phase is approximately the same regardless of whether leaves or roots are inoculated. Evidently the factors that determine the period a virus remains localized are approximately the same in the leaves and roots.

TABLE 4.—*Comparison of the time required for the downward movement of several viruses from an inoculated leaf and from an inoculated area of the roots*

Host	Virus of	Average time (hours) between leaf inoculation and root invasion	Average time (hours) between root inoculation and invasion of the portion of the root system below
Tobacco	Ordinary tobacco mosaic	81	80
do	Ordinary cucumber mosaic	104	120
do	Potato ring-spot	240[a]	288[a]
do	Severe etch	72	80
do	Tobacco ring-spot	210	216[b]
Tomato	Ordinary tobacco mosaic	84	96
do	Potato ring-spot	77	88
do	Severe etch	91	88

[a] Downward movement did not occur within the times indicated when the trials were terminated.

[b] Irregular.

The unidirectional movement of virus in inoculated roots seems to indicate that a mass flow of food materials from the leaves to the growing points of the roots may be concerned in the transportation of the virus. Bennett (8, 9) already has presented considerable evidence indicating that the movement of the viruses of ordinary tobacco mosaic and sugar-beet curly top in tobacco and sugar beets is governed by a mass flow of food materials in the phloem. If this interpretation of virus movement in roots be correct, viruses inoculated at the base of the plant stems, below all leaves, should exhibit the same type of movement as that found in inoculated roots. Accordingly, trials with tomato plants were run in which 1 in. of stem just above the soil level was inoculated by rubbing with the extract of ordinary tobacco-mosaic virus. After appropriate intervals, the stem above the point of inoculation was cut into 5 sections, each 1 in. long. These stem sections and the roots growing from the bottom of the pot 6 inches below the point of inoculation were tested for the presence of virus by inoculating leaves of hybrid plants (Table 5). The virus moved rapidly from the point of inoculation both upward in the stem and downward into the roots. In two of the trials, the virus appeared in the roots before invading the stem above, while in one trial the stem and roots were both invaded within the same 24-hour interval. It appears, therefore, that when the base of the stem is inoculated, the upward movement of the virus is not restricted, as it is in the roots.

The nearly simultaneous movement of virus in both directions from the base of tomato stems is open to several interpretations. If, as Curtis (12) holds, viruses move in both directions in the phloem by a process of activated diffusion, then it must be concluded that this type of movement is radically modified in the roots. On the other hand, if the virus moves in only one

TABLE 5.—*Movement of tobacco-mosaic virus upward and downward from the point of inoculation of the base of the stem of tomato plants under various conditions of illumination*

Treatment of inoculated plant	Position of stem sections tested, inches above or below inoculated section	Number of times[a] virus was recovered in three trials from stem sections taken at positions shown at following daily intervals				
		3 days	4 days	5 days	6 days	7 days
Natural light in greenhouse	Over 4 above	0	0	2	3	3
	2–3 do	0	0	2	3	3
	0–1 do	1	2	2	3	3
	Inoculated section	3	3	3	3	3
	Roots (6 inches below)	0	1	3	3	3
Continually darkened after inoculation	Over 4 above		0	0	0	0
	2–3 do		0	0	0	0
	0–1 do		0	2	3	2
	Inoculated section		3	3	3	3
	Roots (6 inches below)		0	0	0	2
Constantly illuminated after inoculation	Over 4 above		0	2	2	3
	2–3 do		0	2	2	3
	0–1 do		3	3	3	3
	Inoculated section		3	3	3	3
	Roots (6 inches below)		2	3	3	3

[a] One hybrid was used as a test plant for each trial. A trial was recorded as positive if more than one lesion developed.

direction at a time in a mass flow of food materials, then the direction of flow through the base of the stem must alternate during a period of 24 hours. Or, as Kunkel (23) suggests, mass flows in opposite directions may occur in different phloem strands.

On the assumption that reversal in the direction of virus movement in the stem may occur as a result of the daily alternation of light and darkness, trials were conducted in which the plants were kept under constant conditions of illumination and temperature. In one series tomato plants were kept in a dark chamber after inoculating the base of the stem with ordinary tobacco-mosaic virus. In the other series tomato plants were placed in a constantly illuminated chamber (3 to 4 ft. below 6 Mazda 30-watt, 115-volt, daylight fluorescent lamps) at a temperature of 27° C. for 1 day before inoculation of the base of the stem, and were kept under these constant conditions for the duration of the trial. The stem and roots were tested for the virus as in previous trials (Table 5).

Total darkness prevented upward movement of the virus from the base of the stem of the tomato plants. Downward movement into the roots occurred, although not so soon as in plants in the light. Under conditions of total darkness, therefore, unidirectional movement was observed. In constantly illuminated plants, the virus movement was almost simultaneous in both directions. It seems possible, however, that the constant illumination may not have affected the direction of mass flow of food material.

Factors Affecting Virus Movement in Inoculated Roots

If virus movement in inoculated roots occurs in a mass flow of food materials from the leaves into the roots, it should be possible to vary the movement of the virus by treatments designed to vary the flow of food materials. Since the effect of such treatments on the movement of food materials can only be assumed, definite proof of a correlation between food material and virus movement is not possible, although the response of virus movement to different treatments should give some information on the factors involved.

Since the movement of food materials into the roots is dependent upon photosynthesis in the leaves, the absence of light on the aerial parts of the plants may logically be expected to affect the movement of virus in the inoculated roots. Trials of this sort were carried out by inoculating the roots of tomato and tobacco plants, as before, with the ordinary tobacco-mosaic virus, and placing the plants immediately in a dark chamber in the warm greenhouse. At the same time, the effect of darkening on virus movement in the aerial parts was determined by inoculating the third leaf from the top of each of a series of 4 or 5 plants with the ordinary tobacco-mosaic virus, and then keeping the plants in the dark chamber until the roots were tested for virus.

The downward movement of the virus in the inoculated roots was only slightly affected by darkening the plants. Since these trials were run with 2-day intervals between tests for virus, the lesser effects of the treatment on the movement of the virus perhaps were not noticed. In tobacco roots, the ordinary tobacco-mosaic virus moved downward at approximately the same rate when the plants were kept in the dark as when they were kept in the light. In tomato roots, the virus moved downward somewhat more slowly when the plants were darkened. The effect of darkening on the movement of virus in inoculated roots is in sharp contrast to the effect observed when leaves were inoculated, in which case the virus did not reach the roots within the 8-day period of the trials. It seems likely that the roots on darkened plants continued to receive a sufficient flow of food materials from the lower part of the stem to transport the virus downward at a normal rate. The direction of movement of virus in inoculated roots was, therefore, not changed by altering the illumination of the host.

Another attempt to change the direction of virus movement in inoculated roots was made by supplying organic food materials to the roots of darkened plants. Tomato and tobacco plants were placed in the dark chamber for 2 days before inoculating a marked 1-in. section of the roots with tobacco-mosaic virus. The roots below the point of inoculation were then submerged in a solution containing sugar, mineral-salt, and yeast extract, and the plants were kept in the dark. Some growth of microorganisms in the nutrient solution undoubtedly occurred, but this was kept in check by supplying fresh solution in sterile crocks twice daily. The roots were washed quickly in running water each time the solution was changed. The data from a number of trials are presented in table 6. The results show that

TABLE 6.—*Movement of tobacco-mosaic virus in the roots of darkened tobacco and tomato plants when sugar solution was supplied to the roots below the point of root inoculation*

Host	Position of root sections tested, inches above or below inoculated section	Number of times[a] virus was recovered in three trials from root sections taken at positions and time intervals (days) shown after inoculation			
		3	4	5	6
Tobacco	2–3 above	0	1	3	3
	1–2 do	0	1	3	3
	0–1 do	2	2	3	3
	Inoculated section	3	3	3	3
	0–1 below	1	2	2	3
	1–2 do	0	1	1	1
	2–3 do	0	0	1	0
	Over 3 do	0	0	0	0
Tomato	2–3 above	0	0	3	3
	1–2 do	0	0	3	3
	0–1 do	1	1	3	3
	Inoculated section	3	3	3	3
	0–1 below	0	0	1	3
	1–2 do	0	0	0	0
	2–3 do	0	0	0	0
	Over 3 do	0	0	0	0

[a] One hybrid was used as a test plant for each trial. A trial was recorded as positive if more than one lesion developed.

under these circumstances the movement of the virus was upward in the roots and not downward. The effect of supplying a proper nutrient solution to the plant roots appears to offer strong evidence that the direction of virus movement in the roots is determined by the position of the supply of organic food materials.

Concentration of Viruses in Root Extracts

Since the presence of a virus in the roots ordinarily does not result in any injury or macroscopic symptoms, it seems possible that the roots are not so extensively invaded by the virus as are the leaves. Stanley (35) and Price (28) have reported instances in which viruses were less concentrated in the roots than in the leaves of infected plants. These reports appear to show that the roots are less favorable for the multiplication of the virus than are the leaves.

The studies here reported have shown also that root extracts of tobacco and tomato plants infected with ordinary tobacco-mosaic virus contain less active virus than leaf extracts of the same plants. Comparisons were made of 1 in 1000 water dilutions of, respectively, the fresh leaf extract of mosaic plants, the root extract of the same plants, and the extract of roots that had been inoculated directly with ordinary tobacco-mosaic virus. The results are based on inoculations to hybrid tobacco plants, the different extracts paired on opposite halves of the leaves so that each possible pair appeared once within a set of three plants on each of 3 leaf positions. Extracts, prepared at weekly intervals for 5 weeks after the plants were inoculated, were compared in this manner (Table 7).

TABLE 7.—*Relative infectivity of leaf and root extracts diluted 1 to 1000 from tobacco and tomato plants infected with ordinary tobacco mosaic virus*

Host	Sources of extract	Total number of lesions on 6 half-leaves of the hybrid inoculated with extracts of plants infected the following number of weeks				
		1	2	3	4	5
Tobacco	Leaves of infected plants	87	1236	419	511	343
	Roots of infected plants ...	42	353	167	152	[a]
	Inoculated roots	51	334	111	220	230
Tomato	Leaves of infected plants	372	769	596	114	311
	Roots of infected plants ...	222	401	300	245	197
	Inoculated roots	217	417	208	157	194

[a] Roots dead.

Both types of root extracts yielded consistently fewer lesions than the leaf extract. By analysis of variance the difference between the leaf extract and the root extracts was found statistically significant, except that, for some unknown reason, at the 4-week interval the tomato leaf extract gave fewer lesions than did the root extracts. The relative infectivity of the different extracts seemed to remain about the same during the 5-week period.

Effect of Healthy Root Extract on the Ordinary Tobacco-Mosaic Virus

Previous results having shown that the infectivity of the virus extracts from roots is lower than that from leaves, an attempt was made to determine if this situation could be attributed to an inhibitory effect of the root extract of the host on the virus. Due to the ease with which the infectivity of extracts of the ordinary tobacco-mosaic virus can be estimated, this virus was used exclusively in these tests. Fresh tobacco-leaf extracts of the tobacco-mosaic virus were diluted 1 to 500 with tap water. Portions of this diluted extract were then mixed with equal portions of root extracts from several species of healthy plants. As checks, 1-to-1 mixtures were made of the diluted virus extract and leaf extracts of healthy plants, and with phosphate buffer at pH 5.8 to 6.0. This hydrogen-ion concentration was used to approximate the hydrogen-ion concentrations of the plant extracts. The pH of the extracts of the different plants varied somewhat, but that of the root extract was always very nearly the same as that of the leaf extract of the same species. The mixed extracts were allowed to stand 5 minutes before being rubbed on leaves of the hybrid. The 3 extracts were paired with one another on opposite half-leaves, so that every possible pair occurred, in a group of 3 hybrids, once on each of 3 leaf positions. The totals of the local lesions resulting from inoculations with the different extracts are tabulated in table 8.

TABLE 8.—*The effect of healthy leaf and root extracts of various plants on the infectivity of ordinary tobacco-mosaic virus diluted 1 to 500 before treatment*

Source of fresh plant extracts	Total number of lesions on 6 half-leaves of 3 hybrids following treatment of virus with equal volume of		
	Fresh healthy leaf extract	Fresh healthy root extract	Phosphate buffer (control)
Nicotiana tabacum	928	71	728
Lycopersicum esculentum	661	174	598
Cucumis sativus	2	23	444
Helianthus annuus	106	640	806
Dolichos lablab	6	0	290

Healthy tobacco and tomato-root extracts showed a marked inhibitory effect on the ordinary tobacco-mosaic virus. With the other species the root extracts seemed to have no greater inhibitory properties than the leaf extracts. In several cases both leaf and root extracts markedly reduced the infectivity of the extract of tobacco-mosaic virus.

While it cannot be definitely concluded that this inhibitory effect of healthy tobacco-root extract is related to the low infectivity of the root extract of the infected plants, it seems likely that this may be the case. The inhibitory effect of certain plant juices on particular viruses has been noticed fairly frequently (15, 25, 31). That such inhibitors might affect the behavior of the virus was suggested also by Bennett (7), who thought that the inhibitors in the parenchyma of the sugar-beet plant might prevent multiplication of the curly-top virus in tissues other than the phloem.

In attempting to determine the nature of this inhibitory effect of root juice, it is of importance to know whether the virus itself is acted upon or whether the inhibitory action is upon the inoculated leaves, as suggested by Stanley for trypsin (34). The method outlined by Chester (10) for such a determination assumes that if the susceptibility of the host is lowered, the percentage of reduction in the number of lesions will be approximately the same for different dilutions of virus, provided that equal amounts of the inhibitor are present in each dilution. If, on the other hand, the effect is on the virus, then the percentage reduction will be low when an excess of virus is present, and greater as the proportion of virus is decreased.

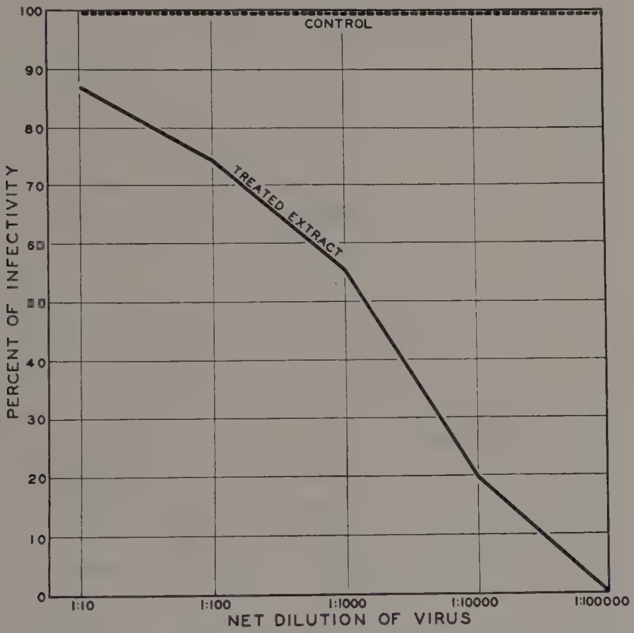

FIG. 5. Percentage of inhibition of tobacco-mosaic virus by healthy tobacco root extract at various dilutions of the virus. The control dilutions contained an equal volume of phosphate buffer. The treated dilutions contained an equal volume of healthy root extract. The control and treatment at each dilution were inoculated to the opposite halves of five leaves of the hybrid plant.

Trials of this sort were run by diluting fresh tobacco-mosaic leaf extract with tap water to 1½ times the final concentration desired. To 2 cc. of each of these dilutions was added 1 cc. of healthy tobacco-root extract. After standing 5 minutes, the mixtures were rubbed on the left half of one leaf of each of 5 hybrid plants, so that each mixture appeared once on each of 5 leaf positions. The controls were prepared by mixing 2 cc. of the virus dilutions with 1 cc. of phosphate buffer at pH 5.6, the hydrogen-ion concentration of the root extract. The control dilutions were rubbed on the half-leaves opposite those rubbed with corresponding dilutions containing root extract. The results of one trial are presented graphically in figure 5. Other trials gave similar results. The effect of the root extract is, according to Chester's interpretation, on the virus, since the percentage of reduction in the number of lesions rises to 100 as the proportion of virus content becomes less. The total numbers of lesions obtained at the dilutions from 1 to 10 up to 1 to 100,000 were, for the control: 2486, 1361, 384, 132, 47, and for the dilutions containing root extract: 2193, 1001, 196, 25, 0.

Thermal-inactivation Point of Viruses in Root Extracts

Since the infectivity of ordinary tobacco-mosaic virus is markedly affected by healthy tobacco and tomato root extracts, experiments were performed to determine if a property of the virus, such as thermal inactivation point, may also be modified when the virus is present in root extracts. Price (29) has shown that dilution slightly lowers the thermal-inactivation point of tobacco-mosaic virus, and it, therefore, seemed likely that the lower infectivity of the root extract might account for the lower thermal-inactivation points observed in preliminary trials. The infectious leaf extract, the control, was, therefore, diluted 1 to 3 with healthy leaf extract in order to lower the virus concentration to approximately that of the root extract. It is recognized that this correction is only a rough approximation, particularly, since some of the viruses used had not been investigated as to their infectivity in root extracts. Judging from Price's results (29), however, only slight differences in thermal-inactivation points are to be expected in dilutions up to 1 to 20 with healthy juice.

The trials were carried out by placing 1 cc. of the freshly extracted root and leaf juice in thin-walled, stoppered test tubes and immersing for 10 minutes in a water bath at the desired temperature. The temperature was controlled to within 0.2° C. In every case, both extracts were heated simultaneously, and, after heating, immediately cooled in running water. Intervals of 4° C. were used in all trials, since consistent differences at ranges of this magnitude should definitely be significant. Inoculations were made to 5 young Havana-seed tobacco plants with all viruses used, except that of ordinary tobacco mosaic, for which the local lesion-producing hybrid was used as a test plant. The data obtained with 4 viruses are summarized in table 9.

TABLE 9.—*Comparison of thermal-inactivation temperatures of different viruses in leaf and root extracts of tobacco*

Virus of	Thermal-inactivation point (° C.), average of 3 trials with temperature intervals of 4° C.	
	Leaf extract	Root extract
Ordinary tobacco mosaic	92	84
Ordinary cucumber mosaic	72	67
Tobacco ring-spot	64	59
Severe etch	60	57

It may be noted from these results that each virus in root extract was inactivated at a temperature 4° C. to 8° C. lower than the virus in the corresponding, diluted leaf extract.

The differences in thermal-inactivation points determined for these viruses in leaf and root extracts seem too large to be due to differences in original concentration of the virus. Some substance or condition peculiar to the root extract evidently reduces the heat tolerance of the virus.

Additional trials were conducted with tobacco-ring-spot virus extracts from four species of plants other than tobacco (*Helianthus annuus, Zinnia elegans, Calendula officinalis, Cucumis sativus*). Again the thermal-inactivation point of the virus in root extracts was 4° C. to 8° C. lower than in leaf extracts in all the hosts used. Apparently this behavior is not characteristic of the roots of any particular plant species, but is more likely quite general in occurrence.

DISCUSSION

The results of this investigation have demonstrated certain differences in virus behavior in roots as compared to behavior in leaves and stems of plants. The most striking difference is the restricted upward movement of virus in directly inoculated roots. Consequently, root inoculation under favorable experimental conditions did not result in leaf symptoms within periods up to 28 days, where leaf inoculations yielded symptoms in 10 days or less. Previous reports (21, 24, 27) of failures to obtain leaf symptoms when the roots were inoculated directly with the virus may be explained largely by the failure of the virus to move upward into the stem of the plant, even though good root infection may have occurred in such trials.

Although roots may be directly infected by viruses, it is questionable whether this mode of infection is of any importance in the occurrence of virus diseases in crop culture. The evidence indicates that leaf symptoms resulting from root infection could occur only relatively late in the life of annual plants, and consequently would be of minor importance. It has been pointed out by others (21) furthermore that from a practical standpoint more abundant opportunities for infection with tobacco-mosaic virus from the soil may occur through leaves and stems than through the roots.

The evidence obtained on the movement of different viruses seems to bear directly on the problem of translocation of organic materials in plants. Bennett (8) has shown that the sugar-beet curly-top virus, in particular, moves through infected plants as if in a mass flow of food materials in the phloem. That a similar mechanism is involved in tomato and tobacco roots is shown rather definitely by the response of the ordinary tobacco-mosaic virus to the presence of a sugar solution below the point of inoculation of the roots. The movement of the virus, although reversed by the treatment, was still unidirectional. This type of movement is a necessary corollary of the mass-flow theory. On the other hand, the movement of tobacco-mosaic virus in inoculated stems of tomato plants is more difficult to correlate with the movement of food materials, since the virus moved relatively rapidly, both upward and downward, within a short space of time. It does not seem logical that in the stem the mechanism governing the movement of virus should be radically different from that operating in the roots. Kunkel (23) has suggested that in tomato stems movement of tobacco-mosaic virus in different phloem strands may account for the apparent simultaneous movement in opposite directions. Anatomical support for this suggestion is found in the presence of internal and external phloem in tomato and tobacco stems. Apparently, in the roots, at least in tobacco (4), only external phloem is present. Thus, in the stem, there exist two more or less separate channels for the transport of organic material, while, in the roots, there is only one. There is yet no evidence supporting the assumption that opposite-directional mass flows occur in the internal and external phloem of tobacco and tomato, but this possibility should be taken into consideration in future studies.

Samuel (32) concluded that the ordinary tobacco-mosaic virus, when inoculated to mature leaves of fairly large tomato plants, moved first to the roots and then to the growing tips of the stems. He suggested that the food materials in the plants might also first move to the roots and then to the growing point of the stem. Kunkel (23) has shown that ordinary tobacco-mosaic virus does not always move downward in tomato stems before moving upward. Since it has been found in the investigation here reported that viruses do not readily move upward in the roots under ordinary conditions, it appears that they could not reach the growing stem tip by moving rapidly upward from the roots. There also seems, therefore, little reason to believe that food materials in rapidly growing annual plants regularly move to the growing tip of the stem by way of the roots.

White's (37) report that inoculation of excised tomato roots *in vitro* with the tobacco-mosaic virus failed to produce infection is of interest in connection with our results on virus movement in roots. One possible explanation is that infection occurred, but that the virus remained localized. A mass movement of food materials may not be expected to occur in roots entirely submerged in a nutrient solution. In the absence of such a movement, which appears to govern virus transportation, it seems likely that the virus might remain localized in the inoculated excised roots.

The conclusion that viruses in root extracts are more readily inactivated by heat than when in leaf extracts is subject to several interpretations. That the virus itself is permanently changed in the root tissues seems unlikely. Bawden (5) has pointed out that the observed thermal-inactivation points of viruses may be influenced by absorption of the virus on precipitates of normal plant proteins formed at certain temperatures. It seems more likely that an absorption of this nature is responsible for the lower thermal-inactivation points observed in root extracts. The results, nevertheless, show that this property of a virus may be modified by the character of the extract carrying the virus, and it is possible that other properties also are modified. For this reason it may be unwise to differentiate viruses on the basis of small differences in their thermal inactivation unless they are grown and tested in the same host extract or medium.

Many new problems for future investigation have arisen during the course of the present investigation on viruses in root tissues. It seems logical to suppose, for example, that heavy virus infections not extending beyond the roots may materially impair the metabolism of the entire plant. With the various techniques now available for measuring physiological processes, it should be possible to determine definitely the effect of root infection on the physiological processes in the host plant. It is likely that not only physical symptoms such as dwarfing may result from root infection, but that more basic processes such as absorption of water and mineral nutrients may be directly influenced.

SUMMARY

The behavior of various viruses in the roots of several host plants has been compared with that of the same viruses in the aerial parts of the plants. Frequency of occurrence of viruses in the roots of systemically invaded plants, susceptibility of roots to direct infection, rate and direction of virus movement in the roots, and influence of root extracts on virus infectivity have chiefly been investigated.

All viruses that invaded systemically the aerial portions of the host plants also were found to invade the roots of the same plants. Root invasion, as a rule, occurred within 5 to 15 days after leaves had been inoculated.

Direct inoculation of roots of tobacco and tomato plants with several different viruses resulted in good root infection, but in no infection of the aerial parts of the same plants. This result is explained on the basis of the direction of virus movement in inoculated roots.

The movement of viruses upward in inoculated roots proved very slow and restricted, whereas virus movement downward in the roots occurred at a relatively rapid rate, corresponding to the rate of downward movement in the vascular tissues of leaves and stems. In contrast to the unidirectional movement observed in inoculated roots, ordinary tobacco-mosaic virus moved rapidly in both directions away from an inoculated area at the base of tomato stems.

Root extracts of tobacco and tomato plants infected with the ordinary tobacco-mosaic virus were found to be less infectious than leaf extracts of the same plants. It was found that root extracts of healthy tobacco and tomato plants had a marked inhibitory effect on the virus of ordinary tobacco mosaic; it appears likely, therefore, that the reduced infectivity of the virus in the roots is not necessarily a consequence of low concentration.

The thermal inactivation points of several viruses formed in the roots of infected plants was 4 to 8° C. lower than the viruses in the leaf extracts from the same plants. It is not improbable, therefore, that other properties of certain viruses may be modified in the roots or root extracts.

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LITERATURE CITED

1. ALLARD, H. A. The mosaic disease of tobacco. U. S. Dept. Agr. Bull. 40, 33 pp. 1914.
2. ———. The mosaic disease of tomatoes and petunias. Phytopath. 6: 328–335. 1916.
3. ———. Further studies of the mosaic disease of tobacco. Jour. Agr. Res. [U.S.] 10: 615–632. 1917.
4. AVERY, G. S. Structure and germination of tobacco seed and the developmental anatomy of the seedling plant. Amer. Jour. Bot. 20: 309–327. 1933.

5. BAWDEN, F. C. Plant viruses and virus diseases. 272 pp. (Leiden.) 1939.
6. BENNETT, C. W. Plant-tissue relations of the sugar-beet curly-top virus. Jour. Agr. Res. [U.S.] 48: 665–701. 1934.
7. ———. Studies on properties of the curly top virus. Jour. Agr. Res. [U.S.] 50: 211–241. 1935.
8. ———. Correlation between movement of the curly top virus and translocation of food in tobacco and sugar beet. Jour. Agr. Res. [U.S.] 54: 479–502. 1937.
9. ———. Relation of food translocation to movement of virus of tobacco mosaic. Jour. Agr. Res. [U.S.] 60: 361–390. 1940.
10. CHESTER, K. S. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic by immune sera. Phytopath. 24: 1180–1202. 1934.
11. CRAFTS, A. S. Movement of viruses, auxins, and chemical indicators in plants. Bot. Rev. 5: 471–504. 1939.
12. CURTIS, O. F. The translocation of solutes in plants. 273 pp. (New York and London.) 1935.
13. DOOLITTLE, S. P. The mosaic disease of cucurbits. U. S. Dept. Agr. Bull. 879, 69 pp. 1920.
14. ———, and F. L. WELLMAN. *Commelina nudiflora*, a monocotyledonous host of a celery mosaic in Florida. Phytopath. 24: 48–61. 1934.
15. DUGGAR, B. M., and J. K. ARMSTRONG. The effect of treating the virus of tobacco mosaic with the juices of various plants. Ann. Mo. Bot. Gard. 12: 359–366. 1925.
16. ESAU, K. Some anatomical aspects of plant virus disease problems. Bot. Rev. 4: 548–579. 1938.
17. FAJARDO, T. G. Studies on the mosaic disease of the bean (*Phaseolus vulgaris* L.). Phytopath. 20: 469–494. 1930.
18. HOLMES, F. O. Local and systemic increase of tobacco mosaic virus. Amer. Jour. Bot. 17: 789–805. 1930.
19. HUTCHINS, L. M. Apparent localization of phony disease virus in the woody cylinder. (Abstract.) Phytopath. 29: 12. 1939.
20. JOHNSON, J. A tobacco hybrid useful for virus studies. Amer. Jour. Bot. 23: 40–46. 1936.
21. ———. Factors relating to the control of ordinary tobacco mosaic. Jour. Agr. Res. [U.S.] 54: 239–273. 1937.
22. ———, and R. W. FULTON. A rare virus disease on tobacco. (Abstract.) Phytopath. 30: 13. 1940.
23. KUNKEL, L. O. Movement of tobacco mosaic virus in tomato plants. Phytopath. 29: 684–700. 1939.
24. LEHMAN, S. G. Contaminated soil and cultural practices as related to occurrence and spread of tobacco mosaic. N. C. Agr. Exp. Stat. Tech. Bull. 46, 43 pp. 1934.
25. MCKINNEY, H. H. Factors affecting certain properties of a mosaic virus. Jour. Agr. Res. [U.S.] 35: 1–12. 1927.
26. MATSUMOTO, T., and K. SOMAZAWA. Immunological studies of mosaic diseases. II. Distribution of antigenic substance of tobacco mosaic in different parts of host plants. Jour. Soc. Trop. Agr. (Japan) 4: 161–168. 1932.
27. MULVANIA, M. Root inoculation with the virus of tobacco mosaic. (Abstract.) Jour. Bact. 19: 23–24. 1930.
28. PRICE, W. C. Acquired immunity to ring-spot in Nicotiana. Contrib. Boyce Thompson Inst. 4: 359–403. 1932.
29. ———. The thermal death rate of tobacco-mosaic virus. Phytopath. 23: 749–769. 1933.
30. ———. Studies on the virus of tobacco necrosis. Amer. Jour. Bot. 25: 603–612. 1938.
31. SALAMAN, R. N. Immunity to virus diseases in plants. Rep. 3rd Int. Congr. Compar. Path. 1: 167–178. 1936.
32. SAMUEL, G. The movement of tobacco mosaic virus within the plant. Ann. Appl. Biol. 21: 90–111. 1934.
33. SMITH, K. M. Studies on a virus found in the roots of certain normal-looking plants. Parasitology 29: 70–85. 1937.
34. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. I. Some effects of trypsin. Phytopath. 24: 1055–1085. 1934.
35. ———. Aucuba mosaic virus protein isolated from diseased, excised tomato roots grown in vitro. Jour. Biol. Chem. 126: 125–131. 1938.
36. WEBB, R. W. Further studies on the soil relationships of the mosaic disease of winter wheat. Jour. Agr. Res. [U.S.] 36: 53–75. 1928.
37. WHITE, P. R. Multiplication of the viruses of tobacco and aucuba mosaics in growing excised tomato root tips. Phytopath. 24: 1003–1011. 1934.

FUSARIUM WILT OF THE MIMOSA TREE (ALBIZZIA JULIBRISSIN)[1]

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INTRODUCTION

The fusarium wilt of the mimosa or silk tree (*Albizzia julibrissin* Durazz.), caused by *Fusarium perniciosum* Hepting,[3] is a highly destructive vascular wilt that, because of its epidemic nature, must be considered a distinct threat to the cultivation of this valued ornamental in the Southeastern States. This disease was first reported in the United States from the Carolinas by Hepting in 1936 (8). It apparently had been present in Tryon, North Carolina, since about 1930, and, by 1936, was found in 3 other localities. In 1939 Hepting (9) reported additional studies of it. He described the associated fungus as a new species, and demonstrated its pathogenicity by the inoculation of young seedlings. Each year since the first report mimosa wilt has been found over an increasingly wider range, and at the close of the 1940 scouting season had been found in scattered localities from Richmond, Virginia, to Hamilton, Georgia. This increase in range is due in part to more complete information and in part to spread of the disease.

The symptoms of mimosa wilt have been described and illustrated adequately by Hepting (9); but, for the sake of clarity, the writer will here review briefly the salient points. Wilt-diseased mimosa trees are characterized by wilting and yellowing of the foliage and, finally, death of the tree. Upon cutting stems of affected plants a brownish discoloration is found in the spring-wood vessels of the outer annual ring. Fungus hyphae are found in these discolored areas, and in several instances conidia have been found within diseased vessels. Conidial cushions of *Fusarium* are frequently formed on the bark of trees that have reached advanced stages of wilt. Of these, probably the most commonly observed was the non-wilt fungus, *Fusarium lateritium,* or some of its forms. At the same time that saprophytic fusaria begin to fruit on the bark of wilt-killed mimosa, the wood often is invaded by ambrosia beetles, which form tunnels both in diseased and healthy wood. Species of insects found include *Xyleborus affinis* Eichh.,

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[2] The writer wishes to express his appreciation to Dr. F. A. Wolf of Duke University, and to Dr. G. H. Hepting of the Division of Forest Pathology for suggestions and help.

[3] Full agreement was found with the description of *Fusarium perniciosum* given by Hepting (9). Much greater variation in culture, however, has been found than was reported previously. Many of the strains vary so much from Hepting's type strain that they would easily be named different varieties or even, species in some cases, were their origin not known. But since it has been shown that these forms may be derived with great facility from one another by saltation, it is not considered necessary to distinguish them as varieties. Almost all forms have in common some degree of pathogenicity to *Albizzia,* as well as exceptionally short 3-septate conidia. If the system of classification proposed by Snyder and Hansen (13) were used, this fungus would be called *Fusarium oxysporum* form *perniciosum* (Hepting), n. comb.

Pterocyclon mali Fitch., and *Pterocyclon fasciatum* Say;[4] *Pterocyclon fasciatum* was the most abundant.

It seemed desirable, because of the seriousness of the mimosa wilt, to gain further information on variation in morphology and pathogenicity of the causal fungus, its host range, life history and means of dissemination, the influence of environmental factors upon the disease, and the origin of the disease. Knowledge of these facts is basic to the development of control measures for this and similar diseases.

CULTURE STUDIES OF CAUSAL ORGANISM

Numerous isolations of *Fusarium perniciosum* and related fungi were made from wilt-diseased mimosas. A selection from these cultures was made for additional study. The origin of these isolates is given in table 1. This includes number, species and cultural class, date of isolation, and source. The numbers are those assigned to the original cultures at the time of isolation and will be used in future reference to these cultures. The isolates of *F. perniciosum* are divided into 3 classes on the basis of cultural characters. These will be explained in detail under cultural studies.

TABLE 1.—*List of fusaria isolated from mimosa trees affected with wilt and used in infection experiments and cultural studies*

Species and cultural class	Assigned culture number	Date of isolation	Source of culture
F. decemcellulare	293	9/22/38	Bark tissue, Wadesboro, N. C.
F. lateritium	282	9/22/38	“ “ , Tryon, N. C.
F. solani martii	109	9/17/38	Vascular tissue, Osceola, S. C.
F. sp.	163	9/17/38	“ “ , Tryon, N. C.
F. perniciosum, appressed class[a]	152	9/17/38	“ “ , Spartanburg, S. C.
	42s1	12/13/38	Sector from number 42, Wisacky, S. C.
	42s2	12/13/38	“ “ “ “ “ “
F. perniciosum, raised mycelial class	126s1	12/13/38	“ “ “ 126, Van Wyck, S. C.
	101as1	12/13/38	“ “ “ 101, Pineville, N. C.
	126s2	12/13/38	“ “ “ 126, Van Wyck, S. C.
	47	9/16/38	Vascular tissue, Wisacky, S. C.
	158	9/17/38	“ “ , Tryon, N. C.
F. perniciosum, raised sclerotial class	37	9/16/38	“ “ , Johnston, S. C.
	1	8/15/38	“ “ , Charlotte, N. C.
	95	9/17/38	“ “ , Polkton, N. C.
	101	9/17/38	“ “ , Pineville, N. C.

[a] The explanation of the arrangement of the isolates of *Fusarium perniciosum* into 3 classes is given under the discussion of culture studies.

Variation

In the present study it was noticed from the first that there was considerable variation among the numerous isolates of the mimosa wilt fungus. Following a modification of systems of classification used by other investigators (3, 18) the isolates were separated into the following 3 groups or classes based on differences in growth characters on 3-per cent malt agar:

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[4] Determinations by M. W. Blackman, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington, D. C.

class 1, appressed, mat mostly slimy without aerial mycelium, occasional cottony tufts of aerial mycelium, vinaceous-purple[5] to white; class 2, raised mycelial, mat raised, felty to cottony, various shades of purple; class 3, raised sclerotial, mat raised, woolly, with sclerotia-like clumps of mycelium and occasionally sporodochia. It is recognized that there are no sharp boundaries between these cultural classes, since certain intermediate forms always are found, and for this reason it is probably not worthwhile to attempt to make many subdivisions of cultural classes. Grouping into at least the above classes, however, is useful as an aid in the interpretation of the observed variations.

In the course of this investigation the various strains of *Fusarium perniciosum* were grown on 7 different agar media, 3 different liquid media, and 2 different solid media.[6] Type of growth was not only different on the various media but varied on the same medium. It was apparent from these studies that the cultural characters of *F. perniciosum* can be completely changed by changing the medium upon which it is grown. Changes in color were particularly noticeable, and it was found that color changes could be brought about by changing the C/N ratio of the media. This work is in agreement with that of Brown and Horne (5) with other fusaria.

Saltation

While comparing the growth of some of the strains of *Fusarium perniciosum* isolated in Petri-dish cultures, sharply defined sectors were observed. Sectoring occurred in monoconidial cultures, as well as in cultures from mass isolates, and it was observed in all cultural classes. Isolates from these sectors usually remained constant, but occasionally there was additional sectoring. The number of cells comprising a spore has no apparent effect on sectoring, since it was as common in monoconidial cultures from 0-septate as from 3-septate conidia. In some cases microscopic, as well as macroscopic, cultural characters of the sectors varied from those of the parent culture. Cultures with abnormally long spores sometimes appeared as sectors arising from cultures having normal-size spores.

Sectoring in culture is known to occur in many genera of fungi, and has been referred to as variation, chimera formation, saltation, mutation, and dissociation by various investigators. Goddard (6) gives an extensive review of this subject. Since the genetic nature of sectoring is unknown, it seems best to use the rather noncommittal word saltation, which was first proposed by Stevens (14). Saltation occurred more often in the mycelial cultural class than in the appressed class. All possible sectoring combinations were found of the 3 cultural groups recognized. It is probable, as Brown (3) points out, that by continued cultivation and separation of saltants a more or less continuous series of forms could be obtained.

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[5] Color designations follow Ridgway (11) for the most part.

[6] Potato-dextrose agar, malt agar, corn-meal agar, prune agar, Brown's standard synthetic agar, Brown's color synthetic agar, Richard's synthetic agar, Tochinai's solution, Fedstov's solution, Richard's solution, mimosa twigs, and rice grains.

Spore Characteristics

Although size of conidia will vary with change in a number of environmental factors, as was pointed out by Harter (7), measurements of about 3,000 conidia representing 54 separate isolates of *Fusarium perniciosum* from widely scattered localities gave figures in close agreement with those of Hepting (9), except in the case of those cultures that produced abnormally long spores by saltation.

Under proper moisture conditions the conidia of *Fusarium perniciosum* germinate throughout a wide range of temperatures. Spores placed on agar in Petri dishes at room temperature (24° C.) germinated readily after a period of about 5 hours. A number of conidia were germinated on agar plates and kept under observation for 18 hours. Five hours after planting, a short germ tube had appeared at each end of the spore. After 8 hours these had barely exceeded the length of the spore. Branching did not begin until 13 hours, when the germ tubes were 15 or more times the length of the spore. Secondary branching did not occur until 17 hours after planting. Anastomoses between the hyphae took place and a thick mat of mycelium was quickly formed.

In 1939 Hepting (9) reported considerable difficulty in obtaining consistent results in inoculation experiments. Inserting the fungus into wounds on stems gave negative results, and only occasionally were inoculations successful when roots were treated in a similar manner. He was successful with very young seedlings planted in inoculated soil, and his conclusions were that "although these results indicate the suspected fungus to be pathogenic, the killing of such young seedlings may be a quite different process from the killing of grown trees." The present experiments were undertaken to establish further proof of pathogenicity, to investigate relative virulence of various cultural strains of the causal fungus, and to determine the host range.

VARIATION IN VIRULENCE

Methods

Variation in virulence of a number of strains of *Fusarium perniciosum* was tested by planting inoculated trees in soil. For comparison some inoculated seedlings were set in tap water.

The inoculation experiments in soil were carried out in the greenhouse at the Bent Creek Experimental Forest of the Appalachian Forest Experiment Station near Asheville, North Carolina. Metal flats made of 26-gauge, galvanized iron with solder-sealed joints, each measuring 12 × 16 × 8 inches, were used as containers. The inner surfaces of these flats were coated with asphalt roofing tar in order to prevent any reaction of the zinc with the fungi. The soil used in these experiments consisted of a mixture of 1 part loam to 3 parts coarse sand that had been sterilized in an autoclave under a pressure of 15 lb. for 1 hour. The following 10 isolates of *Fusarium perniciosum* were used: numbers 158, 126s1, 126s2, 47, 101, 101as1, 152, 42s1,

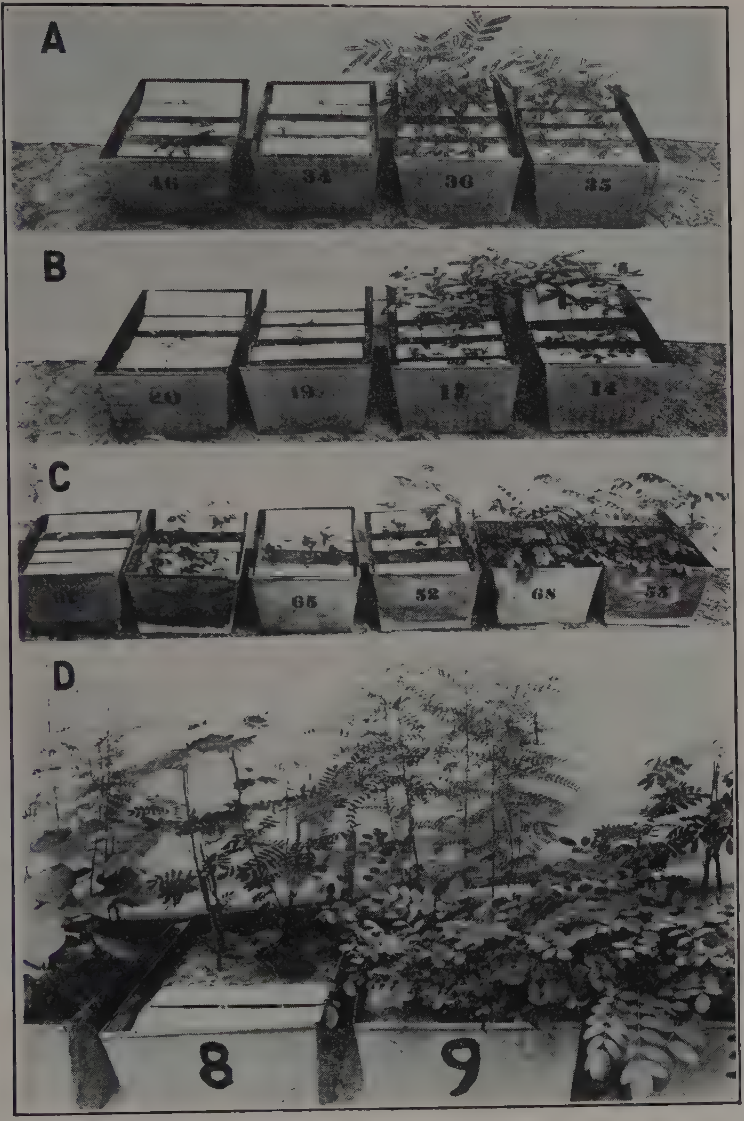

FIG. 1. Results of inoculating various tree species 60 days after inoculation. A. Flats 35 and 36, each contain 12 *Albizzia julibrissin* trees inoculated with *Fusarium decemcellulare;* flats 34 and 46 contain similar trees inoculated with *F. perniciosum.* B. Flats 11 and 14 each contain 12 noninoculated *A. julibrissin* trees, and flats, 19 and 20 contain similar trees inoculated with *F. perniciosum.* C. Flat 67 contains 9 *A. julibrissin* trees in the front and 4 *A. lebbek* trees in the back inoculated with *F. perniciosum;* flat 54 contains similar trees, not inoculated. Flat 65 contains 9 *Cercis canadensis* trees in the front, and 4 *A. lophantha* trees in the back, inoculated with *F. perniciosum;* flat 52 contains similar trees, not inoculated. Flat 68 contains 9 *Robinia pseudoacacia* trees in the front and 9 *Gleditsia triacanthos* trees in the back inoculated with *F. perniciosum;* flat 53 contains similar trees, not inoculated. D. *C. canadensis, A. lebbek, G. triacanthos, A. julibrissin,* and *R. pseudoacacia* trees inoculated with *F. perniciosum. A. julibrissin* trees dead and others healthy.

42s2, and 1. Other fungi tested included number 109, *F. solani martii,* number 163, *F.* sp., number 293, *F. decemcellulare,* and number 282, *F. lateritium.* The sources of these cultures have been indicated in table 1.

The roots of the plants to be inoculated were dipped into a water suspension of the *Fusarium* made from cultures grown in a Tochinai liquid medium. This method of inoculation, developed by Wellman (17), was found most satisfactory and was used throughout the research here reported. The fungus suspension was prepared as follows: the excess liquid was poured off, since it was found in preliminary trials to be very toxic; water was then added to the mat which was then beaten with an eggbeater until a dense suspension of spores and hyphae was formed. For comparison with this method other inoculations were made by the addition to the soil of the *Fusarium* grown on rice grains.

Seedlings of *Albizzia julibrissin* of two age classes were used. The first were 3-year-old seedlings grown from seed collected at Tryon, N. C., and the second were 1-year-old seedlings grown from seed collected at Belmont, N. C. Six inoculated seedlings of each age class were planted in duplicated flats for each strain of the fungus employed. A portion of this experiment is illustrated in figure 1, A and B. The plants were watered sufficiently to keep the soil moisture within a range optimum for growth, since excessively dry or wet soils inhibit the expression of diseases of this type (17). It was not possible to control temperature, but records were kept of both air and soil temperatures throughout the course of these experiments. The first appearance of disease symptoms and their daily progress with each seedling were recorded.

To determine whether comparable results could be obtained by using water instead of soil, a water-culture experiment was carried out. In this experiment 1-year-old seedlings of mimosa, from the same source as those used in the inoculation experiment in soil mentioned above, were similarly inoculated and placed in test tubes filled with tap water.

Results and Discussion

To portray the progress of the wilt in these inoculation experiments a detailed account of a single seedling will be given. This seedling belonged to the 3-year-old age class, which was inoculated with *Fusarium perniciosum,* isolate 126s2. At time of inoculation this plant, like all others, had not leafed out. About a week after inoculation, however, the leaves began to expand. Thirty-eight days after inoculation the first external symptom of disease was noted; 4 pinnae showed drooping and some wilting; 4 days later these had become dried and shrivelled, 3 more pinnae had begun to wilt, and the tree was completely wilted by the following day. Thus, 6 days after the first symptom appeared, the tree had completely wilted. This was followed in a few more days by defoliation and, finally, death. Upon dissection this tree showed vascular discoloration in the tap root and lateral roots whence the original fungus strain was reisolated.

TABLE 2.—*Development of mimosa wilt in mimosa trees inoculated with different isolates of F. perniciosum*[a]

Days after inoculation	Trees diseased, by cultural class, using liquid culture inoculum														Trees diseased, using rice-grain inoculum			
	Raised sclerotial cultural class				Raised mycelial cultural class													
	Isolate No. 1		Isolate No. 101		Isolate No. 101as1		Isolate No. 126s1		Isolate No. 158		Isolate No. 126s2		Isolate No. 47		Isolate No. 47		Isolate No. 101	
	3-yr.[b]	1-yr	3-yr.	1-yr.	3-yr.	1-yr.	3-yr.	1-yr.	3-yr.	1-yr.	3-yr.	1-yr.	3-yr.	1-yr.	3-yr.	1-yr.	3-yr.	1-yr.
Number	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
30	0[c]	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0[d]
35	0	0	0	0	0	0	17	0	17	0	0	0	17	0	0	0	0	0
40	42	0	8	0	33	0	42	0	25	0	25	0	42	0	17	0	0	0
45	50	0	42	0	58	0	92	0	83	0	75	8	83	8	33	0	0	0
50	58	8	58	0	67	0	92	8	92	0	100	17	83	17	33	0	8	0
55	67	8	67	8	83	8	92	33	100	8	100	25	100	17	42	0	17	0
60	92	42	75	8	92	25	92	42	100	17	100	33	100	17	50	0	42	0
65	92	50	83	17	92	33	100	42	100	33	100	58	100	58	67	0	58	0
70	100	58	83	17	100	50	100	50	100	42	100	67	100	58	67	8	58	0
75	100	83	92	33	100	67	100	67	100	42	100	83	100	67	67	8	67	0
80	100	100	100	75[e]	100	92[e]	100	92[f]	100	67[e]	100	100	100	75[e]	75[g]	17[h]	67[h]	50[g]

[a] Inoculations performed on April 24, 1939. Trees of both age classes inoculated with *F. solani martii*, isolate number 109, *F.* sp., isolate number 163, *F. lateritium*, isolate number 282, *F. decemcellulare*, isolate number 293, and appressed strains of *F. perniciosum*, isolates numbers 152, 42s1, 42s2, as well as uninoculated control trees, remained healthy throughout the course of this experiment and are omitted from the table.

[b] Age of seedlings.

[c] Percentage based on 12 trees in each column.

[d] Percentage in this column based on 4 trees.

[e] Trees 100 per cent diseased 90 days after inoculation.

[f] Trees 100 per cent diseased 85 days after inoculation.

[g] Trees 100 per cent diseased 95 days after inoculation.

[h] Trees 100 per cent diseased 110 days after inoculation.

Other trees that became diseased had similar histories, but differed somewhat in rate of wilting.

On some of the seedlings another type of wilting was observed. Here, the abscission layer was apparently formed early with consequent shedding of the leaves in a partially wilted condition. In a few cases, small orange sporodochia of the causal fungus were found at the ground line of a few small inoculated seedlings. In figure 1, A, the healthy appearance of the trees inoculated with *Fusarium decemcellulare,* a fungus isolated from the bark of dying mimosa, can be contrasted with the wilted and dead trees inoculated with a virulent strain of *F. perniciosum.* In figure 1, B, the trees in flats 20 and 19 were inoculated with *F. perniciosum* and those in flats 11 and 14 were controls.

The data from these inoculation experiments are summarized in table 2. Since those plants inoculated with *Fusarium solani martii, F.* sp., *F. lateritium, F. decemcellulare,* and appressed strains of *F. perniciosum,* as well as the noninoculated control plants, all remained healthy, they are omitted from the body of the table. *F. solani martii* and *F.* sp. were isolated from vascular tissue of trees in advanced stages of wilt and probably should be considered as secondary invaders.

Among the different isolates of *Fusarium perniciosum* it soon became apparent that pathogenicity was greatest among the isolates with raised mycelial cultural characters and least among those showing appressed growth. This is in agreement with the results of Wellman and Blaisdell (18), who worked with the tomato-wilt *Fusarium,* as well as those of other investigators. They found that pathogenicity decreased from the raised mycelial cultural class to the appressed class.

Twenty-five per cent of the 24 1-year-old seedlings and 84 per cent of the 24 3-year-old seedlings inoculated with 2 raised sclerotial strains were killed within 60 days of inoculation; the corresponding figures for the 60 seedlings inoculated with the 5 raised mycelial strains were 27 and 96 per cent, respectively; none of the seedlings inoculated with the 3 appressed strains showed any evidence of disease. The difference in pathogenicity between the 1- and 3-year-old seedlings is probably due in part to the different seed sources, since experiments in progress indicate even greater differences in pathogenicity with variation in seed source, and in part to the fact that the 1-year-old seedlings had been grown the previous winter in the greenhouse and were in full leaf at the time of inoculation.

In order to determine whether or not there was a significant difference between the rate of wilting as caused by the raised sclerotial and raised mycelial cultural classes, the data on the number of 3-year-old seedlings wilted were subjected to the analysis of variance. The results of the analysis showed that there were mathematically significant differences between the rate of wilting of these two cultural classes. The curves in figure 2, A, show these differences graphically. The rate of wilting was more rapid with strains belonging to the raised mycelial cultural class than with the raised

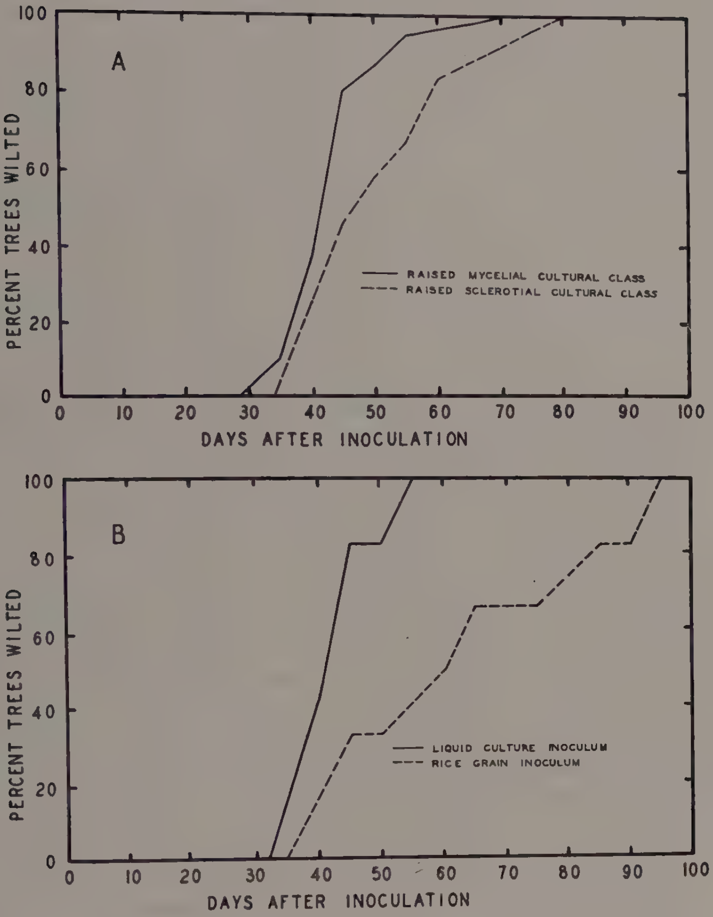

FIG. 2. A. Comparative rate of wilting of 3-year-old trees of *Albizzia julibrissin* inoculated with raised-mycelium or raised-sclerotium isolates of *Fusarium perniciosum;* raised mycelium-culture class based on 60 trees, and raised sclerotium-culture class based on 24 trees. Curves of the appressed-culture class are not included, since all trees remained healthy. B. Comparative rate of wilting of 3-year-old trees of *Albizzia julibrissin* inoculated with isolate number 47 of *Fusarium perniciosum* as liquid culture inoculum and rice-grain inoculum. Each curve based on 12 trees.

sclerotial class. These cultural classes may be arranged in order of virulence as follows:

1. Raised mycelial cultural class; highly virulent.
2. Raised sclerotial cultural class; moderately virulent.
3. Appressed cultural class; nonvirulent.

There was a marked difference in the incubation period between the two methods of inoculation with the two strains on which this was compared. Using the fungus growing on rice grains as the inoculum, complete wilting was 30 to 40 days later than that resulting from the fungus grown in liquid culture. This is illustrated graphically in figure 2, B, for isolate number 47.

Table 3 shows the results of the water-culture inoculation. It will be noted here that the two other species of vascular fungi, No. 163, *Fusarium* sp., and No. 109, *F. solani martii*, caused 33 per cent and 50 per cent wilt, respectively, under these conditions, while in soil they did not produce wilt. Neither the trees inoculated with the bark fusaria, *F. lateritium* and *F. decemcellulare,* nor the control trees showed any evidence of wilt. The cultures in the appressed cultural class showed 60 per cent of the trees wilted in water culture as against none wilted in soil. The raised sclerotial class caused 78 per cent of the seedlings to be wilted, while the raised mycelial type caused 82 per cent. During this same 60-day period the 1-year-old seedlings in the inoculation experiment in soil (table 2) caused 25 per cent and 27 per cent wilted trees, respectively, for these cultural classes.

It is noticeable that certain forms that caused no wilt in inoculations in soil produced some wilt in water. Thus the water method, although somewhat more readily usable, seems not to be comparable with the soil method. Its use with the mimosa wilt, therefore, is not recommended.

TABLE 3.—*Susceptibility of 1-year-old mimosa seedlings to culturally different isolates of Fusarium perniciosum and other fusaria isolated from mimosa using the water-culture method of inoculation*[a]

Fungus species and cultural class	Isolate number	Trees used	Trees wilted 60 days after inoculation
		Number	*Per cent*
None	Control	3	0
Fusarium sp.	163	3	33
F. solani martii	109	4	50
F. lateritium	282	5	0
F. decemcellulare	293	4	0
F. perniciosum, appressed cultural class	152	5	80
	42s2	4	100
	42s1	4	0
Total		13	60
F. perniciosum, raised sclerotial cultural class	1	5	80
	101	4	75
Total		9	78
F. perniciosum, raised mycelial cultural class	101as1	3	66
	126s1	5	100
	158	5	80
	126s2	4	75
Total		17	82

[a] Trees inoculated April 25, 1939.

SUSCEPTIBILITY OF DIFFERENT HOST SPECIES

Methods

The susceptibility of different leguminous host species to the mimosa wilt *Fusarium* was tested by a number of experiments. In experiment 1 the relative susceptibility of 1- and 3-year-old seedlings of *Albizzia julibrissin, Albizzia lebbek* Benth., *Robinia pseudoacacia* L., *Gleditsia tri-*

acanthos L., and *Cercis canadensis* L. was tested. Trees of these species were inoculated and set in metal flats of soil, according to the procedure previously described.[7] In this instance strains of *Fusarium perniciosum* numbers 47 and 101 were used. With each of these strains 18 seedlings of *A. julibrissin, R. pseudoacacia, G. triacanthos,* and *C. canadensis,* and 8 seedlings of *A. lebbek* were inoculated, but several did not survive transplanting and were omitted from number inoculated in table 4. The seedlings inoculated with each strain were divided into 2 equal lots, and each lot planted in one-half of one of the metal flats so that each flat contained 2 different tree species. A similar set of noninoculated trees was set up as a control. All of the seedlings used in this experiment, except those of *A. julibrissin* were obtained from nurseries.

In experiment 2 this work was repeated exactly as above, except that 2-month-old seedlings were used, the seedlings were grown locally in sterilized soil, and *Albizzia lophantha* Benth. was added. Additional inoculations comparing the wilting of *Albizzia lophantha* and *A. julibrissin* were made in experiment 3. In experiment 4, seedlings of *A. thorelii* Pierre, obtained from the United States Department of Agriculture Plant Introduction Garden, Glenn Dale, Maryland, and *Mimosa pudica* L. were inoculated.

In experiment 5, seedlings of *Albizzia julibrissin* and *A. lebbek* were grown from seed in test tubes on sterile malt-agar slants. The seeds were sterilized in concentrated sulphuric acid before planting. When the seedlings were 10 days old, strains 47 and 101 of *Fusarium perniciosum,* as well as strain 109 of *F. solani martii* were placed on the agar of different sets of tubes. Five tubes, each with a seedling, were used for each fungus strain or species and for the set of controls. Small bits of mycelium from stock cultures of the different fungus strains were transferred to the surface of the agar near the seedling, so that at no time were the root systems of these seedlings injured in any way. This experiment was designed to see if *F. perniciosum* can invade healthy uninjured roots.

Results and Discussion

The results of these experiments are summarized in table 4. In figure 1, C, the results with the 2-month-old seedlings can be seen, while figure 1, D, shows a view of some of the trees of the 1- and 3-year-old age classes.

Of the Leguminoseae thus far tested, only members of the genus *Albizzia* have proved susceptible to *Fusarium perniciosum. Robinia, Gleditsia, Cercis,* and *Mimosa* seem resistant. This is similar to the results of Bohn and Tucker (2) whose experiments with the *Fusarium* wilt of tomato indicate that the parasitism of *Fusarium lycopersici* is limited to the genus *Lycopersicon. Albizzia lophantha* and *A. lebbek* have proved susceptible to this disease in addition to *A. julibrissin. A. thorelii* to date has appeared to be resistant, but further tests of this species are needed before definite

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[7] See pages 601, 602.

TABLE 4.—*Susceptibility of different leguminous tree species to two isolates of Fusarium perniciosum*

Experiment no.	Age of stock and inoculation method	Tree species	Isolate no. 47		Isolate no. 101		Control		Remarks
			Trees inoc.	Trees wilted	Trees inoc.	Trees wilted	Trees used	Trees wilted	
1	1- and 3-yr. seedlings, roots inoculated, transplanted to sterile soil.	*Albizzia julibrissin*	18	18	18	18	18	0	Inoc. 4/13/39. All *A. julibrissin* wilted after 90 days, first wilt in 29 days. *A. lebbek* wilted in 95 days, first symptom after 48 days. Original fungus was reisolated from all wilted trees.
		A. lebbek	8	3	8	0	8	0	
		Cercis canadensis	13	0	18	0	17	0	
		Robinia pseudoacacia	17	0	17	0	15	0	
		Gleditsia triacanthos	18	0	18	0	16	0	
2	2-month seedlings, roots inoculated, transplanted to sterile soil	*A. julibrissin*	18	18	18	18	18	0	Inoc. 6/14/39. *A. lophantha* first wilt in 12 days, all wilted in 21 days. *A. lebbek* first wilt after 21 days, all wilted in 47 days. *A. julibrissin* first wilt in 21 days, all wilted in 27 days. Some trees after complete defoliation formed a few new leaves.
		A. lebbek	8	8	8	0	8	0	
		A. lophantha	8	8	8	0	8	0	
		C. canadensis	18	0	18	0	18	0	
		R. pseudoacacia	18	0	18	0	18	0	
		G. triacanthos	18	0	18	0	18	0	
3	2-month seedlings, roots inoculated, transplanted to sterile soil.	*A. julibrissin*	12	12	12	12	6	0	Inoc. 7/12/39. *A. julibrissin* first wilt in 33 days, all others in 39 days. *A. lophantha* first wilt in 11 days, all others after 39 days.
		A. lophantha	21	19	18	0	9	0	
4	1-yr. seedlings, roots inoculated, transplanted to sterile soil.	*A. thorelii*	4	0	4	0	4	0	Inoc. 10/12/39. No wilt after 12 months.
		Mimosa pudica	2	0	2	0	2	0	
5	10-day seedlings, grown on agar and inoculated *in situ*.	*A. julibrissin*	5	5	5	5	5	0	Inoc. 5/21/39. With isolate no. 109, 5 out of 5 trees wilted.
		A. lebbek	5	5	0	......	5	0	

conclusions can be drawn. Tests in progress with *A. kalkora* indicate that this species is also somewhat susceptible to *F. perniciosum*. *A. lophantha* is more susceptible than *A. julibrissin* to strain No. 47 and apparently is resistant to strain No. 101, while *A. lebbek* is slightly susceptible to strain No. 47 and seems completely resistant to strain No. 101.

Experiment 5, in which seeds were sown on agar in test tubes, was set up with the hope of determining if wilting could be produced when the root systems had not been wounded or injured in any way. Wilting was produced readily following inoculation, and results comparable with the soil inoculation experiments were obtained, except that wilting was also caused by *Fusarium solani martii,* a fungus that did not produce wilt in the soil inoculation experiments. The conditions in these test-tube cultures were probably more favorable for the invasion of host tissue than in nature. Valleau and Johnson (15) tested seedlings of a number of crop plants with a large number of *Fusarium* species using this method and found that seedlings were killed by *Fusarium* species that, in nature, never cause injury on mature plants.

LIFE HISTORY

Source and Dissemination of Inoculum

By means of artificial-inoculation experiments it has been found that either bits of mycelium or conidia of *Fusarium perniciosum* may serve as inoculum. As the result of microscopic studies it was concluded that, in nature, spore production seems rather meager, so that conidia appear to be of minor importance in the spread of this disease.

Among the factors that might be concerned with the dissemination of this fungus, probably the most outstanding are those traceable to man. Locally, the fungus might spread from tree to tree by growth from root to root and by growth through organic matter rather than appreciably through mineral soil. A number of attempts were made to isolate the pathogen from mineral soil taken from the vicinity of diseased mimosa trees in Tryon, N. C.; in no case was *F. perniciosum* recovered. In some instances water may spread the fungus. Aside from its dissemination through movement of infested soil during rains (9), it may be carried longer distances in streams and rivers. The principal means of dissemination of *F. perniciosum* to widely separated localities is undoubtedly through the transportation of infested soil or infected plant parts. Hepting (9) points out that the transplanting not only of mimosa trees but also of any plants that have been growing in infested soil may spread the disease.

The possibility of insects being important carriers of the fungus deserves consideration, since it has been possible to isolate the causal agent of the mimosa wilt from both adults and larvae of ambrosia beetles and from their tunnels and frass. Although certain species of these beetles have been shown to carry the wilt fungus, they commonly attack only weakened or dying trees and are very rarely known to attack healthy ones. Moreover.

the fact that attempts to produce the disease through trunk inoculations have proved unsuccessful (9) makes it seem doubtful that the beetles would have induced the disease if they had carried the pathogen to a healthy tree. Therefore, they are not regarded as of appreciable importance in the dissemination of the mimosa wilt.

Infection Court

The mimosa-wilt *Fusarium* apparently enters roots either through wounds or directly through root hairs or the epidermis of the small roots. Twelve hours after a spore suspension had been placed on the roots of small mimosa seedlings grown between blotting papers, germ tubes were observed penetrating the root hairs. Twenty-four hours later the fungus had entered several epidermal cells. In some cases typical damping-off symptoms were observed on very young seedlings, and the strain of *F. perniciosum* used in the inoculation was reisolated from the disintegrated basal stem tissue. In addition, the meristematic tissue might serve as an infection court, since Smith and Walker (12) in their cytological study of cabbage plants and Fusarium yellows found that hyphae only occasionally entered through root hairs or epidermal cells. The important infection courts in their case were the embryonic region and region of elongation.

Disease Development

The development of the mimosa wilt in *Albizzia julibrissin* follows closely the description given by Brown (4) for wilts in general. The parasite enters the root system and traverses the cortex to the vascular system where it reaches a striking development. The proper functioning of the vascular system is interfered with by plugging effects and probable toxic substances excreted by the fungus. As a result the foliage wilts and the whole plant dies. After *Fusarium perniciosum* enters the root hairs and smaller roots there is an incubation stage of about 30 days in the case of young seedlings, and probably longer with older more mature trees, before the first external symptom is visible. The fungus enters the tap roots from the smaller lateral roots. In a number of cases the vascular discoloration has been traced from small lateral roots into the tap root whence it extends both upward and downward in the vascular system. It is probable that infection most commonly occurs in the late spring and early summer, since, in early stages of wilt, hyphae and brown vascular discoloration are found only in the spring-wood vessels of the outer annual ring. Thus the disease may be more in evidence in the summer and early fall. The fungus eventually kills the tree, and the roots decay, after which the fungus may be liberated into the soil.

TEMPERATURE RELATIONS

The incidence of fusarium wilts is affected by a number of soil factors, including temperature, moisture, texture, organic matter, reaction, and chemical composition. Of these, soil temperature in particular has been shown to have a pronounced influence on the incidence of fusarium wilts (10).

Methods

For the determination of the relation of temperature to fungus growth, the increase in diameter of cultures of the organism on agar plates has been widely used. Although some workers have employed increase in weight of the fungus on liquid media, the validity of the plate method is substantiated by the agreement between colony growth and the incidence of disease curves as shown by Jones *et al.* (10).

The influence of temperature on growth rate of a number of strains of *Fusarium perniciosum, F. solani martii,* and an unknown species of *Fusarium* from *Albizzia* was determined with the use of Brown's synthetic medium. The medium was poured in 90 cm. Petri dishes, about 15 cc. per dish. Twelve isolates were employed. These were divided among various strains and species as follows: Two isolates of *F. solani martii,* 1 isolate of *F.* sp., and 9 isolates of *F. perniciosum* with 1 in the appressed-culture class, 3 in the raised mycelium-culture class, and 5 in the raised sclerotium-culture class. Single-spore isolations were made from each of these 12 isolates and small bits of mycelium of approximately equal size from young, vigorously growing colonies were used to plant test plates for each strain at each temperature.

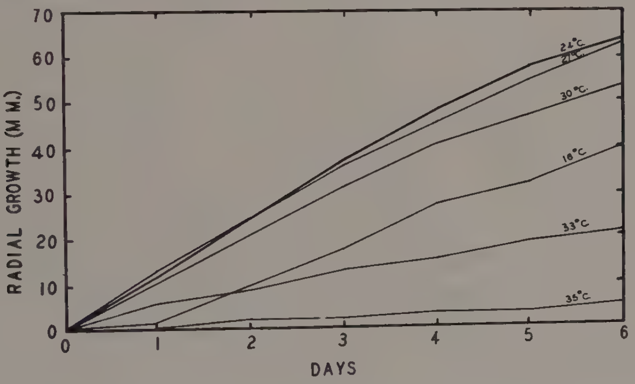

FIG. 3. Curves showing average radial growth of *Fusarium perniciosum* for a period of 6 days at different temperatures on Brown's synthetic medium.

Three plates were prepared for each fungus for each of the temperatures studied. These plates were placed in constant-temperature chambers held at 10, 16, 24, 27, 30, 33, and 35 degrees C., within ± 0.5° C., and incubated for 6 days, when, at the optimum temperature, the colonies were approaching the edge of the plates. The average diameter of each of the colonies was determined at 24-hour intervals.

Results and Discussion

The radial growth of *Fusarium perniciosum* during a 6-day period at different temperatures is shown in figure 3. *F. perniciosum* has a rather sharply defined optimum temperature of about 25° C., with a maximum slightly over 35° C. Although no measurable growth occurred at 10° C., this temperature is not the absolute minimum for development. Cultures kept at 0 and 2° C. are not injured, and over a long period of time may show some growth activity. Although very little growth occurred at 35° C., the fungus was not injured, since, at the close of the experiment, the cultures were removed to room temperature, where they at once resumed growth at the rate normal for this temperature.

Growth of *Fusarium solani martii* and *F.* sp. was somewhat different from that of *F. perniciosum*. *F. solani martii* had an optimum temperature of 27° C. and a maximum of about 37° C. Otherwise, growth curves were similar to those of *F. perniciosum*. *F.* sp. was characterized by having a much slower growth rate than the other two fungi. These growth curves for *F. perniciosum* are similar to those for other vascular wilt fusaria, with which incidence of disease has been shown to be closely correlated (10).

POSSIBLE ORIGIN OF THE DISEASE

There are several ways in which this disease may have originated. The causal fungus may have been present as a weak parasite or saprophyte on some other host plant until it came in contact with the mimosa; or the disease may be caused by some variation or saltation from some common *Fusarium* that has become adapted to the mimosa. To date, with the species tested, no species outside of the genus *Albizzia* has been found susceptible to *F. perniciosum*. If the fungus were a common saprophyte on other hosts, the disease would be expected to be much more widely distributed than it is. The work of Armstrong (1), in which he indicates that the fusarium wilt of cotton and tobacco apparently are caused by the same organism, is of interest in this connection. In view of the great variability of the mimosa-wilt fungus and the facility with which it sectors and saltates, it seems highly possible that this parasite may have arisen as a saltant from some other closely related *Fusarium* of the section Elegans, group Oxysporum. There is no direct evidence, however, in the present case to show that *F. perniciosum* has evolved from some related form.

Another possibility that, because of the epidemic nature of this disease, seems most plausible is that the fungus has been introduced from some foreign land. The disease of *Albizzia julibrissin* reported from Russia (16) is in all probability identical with that under consideration in this report; unfortunately, however, a cultural study of the causal agent of the disease in Russia has never been made. Consequently, positive proof of its identity is lacking. Because of its rather sudden appearance in both Russia and the United States, however, the mimosa wilt may have been introduced into both places. Hepting (9) advances this as the most plausible explanation of the origin of the mimosa wilt. It is possible that *F. perniciosum* originally was present as an endemic pathogen on some other species of *Albizzia* and was introduced into the United States and Russia on this species. The greater resistance of *Albizzia lebbek* and *A. thorelii* lends support to this hypothesis. There are about 40 species of *Albizzia* and many closely related genera distributed throughout the tropical regions of the world; and it is possible that some one or more of these species normally harbors the wilt fungus as a weak parasite.

SUMMARY

The vascular wilt of mimosa (*Albizzia julibrissin* Durazz.), caused by *Fusarium perniciosum* Hepting, was studied. In the United States it has been found in Virginia, North Carolina, South Carolina, and Georgia, where it has destroyed numerous ornamental trees. A similar disease has been described from Russia.

Cultural studies of the fungus isolated from wilt-diseased trees show that there is considerable variation among the numerous isolates. These have been divided into 3 cultural classes based on character of growth in culture, and are designated: (1) appressed, (2) raised mycelial, and (3) raised sclerotial.

The effect of different media on growth of this fungus was studied. The type of growth was found to vary with the media used.

Well-defined sectors were observed in a number of the cultures of *Fusarium perniciosum.* This phenomenon of saltation occurred in all cultural classes.

The pathogenicity studies were divided into two groups: (1) the relative virulence of different cultural variants of the causal fungus, and (2) susceptibility of different tree species. With soil-inoculation experiments it was noted that variation in virulence was related to variation in cultural characters. The cultural classes, arranged in order of virulence, follow: (1) raised mycelial cultural class, highly virulent; (2) raised sclerotial cultural class, moderately virulent; and (3) appressed cultural class, nonvirulent. Water-culture inoculations did not give results in entire agreement with soil inoculations. In this case all forms produced a greater degree of wilting than they did when used in soil inoculations.

In addition to *Albizzia julibrissin,* the susceptibility of *A. lebbek* Benth., *A. lophantha* Benth., *A. thorelii* Pierre, *Robinia pseudoacacia* L., *Gleditsia triacanthos* L., *Cercis canadensis* L., and *Mimosa pudica* L. was tested. It was found that *A. lebbek* was more resistant to a highly virulent strain of *Fusarium perniciosum* than was *A. julibrissin,* and was unaffected by another less virulent strain. *A. lophantha* is more susceptible than *A. julibrissin* to the highly virulent strain tested, but is apparently resistant to certain other strains pathogenic to *A. julibrissin.*

The life history of *Fusarium perniciosum* is briefly as follows: The fungus spreads on or through the soil. When the inoculum comes in contact with mimosa roots it enters through epidermis or root hairs, traverses the cortex to the vascular system, where its development interferes with the proper functioning of this tissue, and, as a result, the foliage wilts and the plant dies. Finally the roots decay, liberating the fungus into the soil again.

The temperature studies of the growth of the fungus in culture show that *Fusarium perniciosum* has a well-defined optimum around 25° C. and a maximum slightly over 35° C. The minimum is not so well-defined, but is somewhat below 10° C.

While the origin of this fungus is unknown, it may have originated as a saltant from some common soil *Fusarium*, or it may have been introduced, possibly on some species of *Albizzia* other than *A. julibrissin*.

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LITERATURE CITED

1. ARMSTRONG, G. M. Fusarium wilt of cotton and tobacco apparently caused by the same organism (Abstract). Phytopath. **30**: 1–2. 1940.
2. BOHN, G. W., and C. M. TUCKER. Studies on Fusarium wilt of the tomato. I. Immunity in *Lycopersicon pimpinellifolium* Mill. and its inheritance in hybrids. Mo. Agr. Exp. Stat. Res. Bull. 311, 82 pp. 1940.
3. BROWN, W. Studies in the genus *Fusarium*. VI. General description of strains together with a discussion of the principles at present adapted in the classification of *Fusarium*. Ann. Bot. **42**: 286–304. 1928.
4. ————. The physiology of host-parasite relations. Bot. Rev. **2**: 236–281. 1936.
5. ————, and A. S. HORNE. Studies in the genus *Fusarium*. III. An analysis of factors which determine certain microscopic features of *Fusarium* strains. Ann. Bot. **40**: 203–221. 1926.
6. GODDARD, M. Studies on variation in *Gibberella Saubinetii* (Mont.) Sacc. (*Fusarium graminearum* Schwabe). Ann. Mo. Bot. Gard. **26**: 99–164. 1939.
7. HARTER, L. L. Influence of light on the length of the conidia in certain species of *Fusarium*. Amer. Jour. Bot. **26**: 234–243. 1939.
8. HEPTING, G. H. A destructive disease of the mimosa tree in the Carolinas. Pl. Dis. Rptr. **20**: 177–178. (Mimeo.) 1936.
9. HEPTING, G. H. A vascular wilt of the mimosa (*Albizzia julibrissin*). U. S. Dept. Agr. Circ. 535, 10 pp. 1939.
10. JONES, L. R., J. JOHNSON, and J. G. DICKSON. Wisconsin studies upon the relation of soil temperature to plant disease. Wis. Agr. Exp. Stat. Bull. 71, 144 pp. 1926.
11. RIDGWAY, R. Color standards and color nomenclature. 43 pp. (Washington.) 1912.
12. SMITH, R., and J. C. WALKER. A cytological study of cabbage plants in strains susceptible or resistant to yellows. Jour. Agr. Res. [U.S.] **41**: 17–35. 1930.
13. SNYDER, W. C., and H. N. HANSEN. The species concept in *Fusarium*. Amer. Jour. Bot. **27**: 64–67. 1940.
14. STEVENS, F. L. The *Helminthosporium* foot-rot of wheat, with observations on the morphology of *Helminthosporium* and on the occurrence of saltation in the genus. Ill. Nat. Hist. Surv. **14**: 77–184. 1922.
15. VALLEAU, W. D., and E. M. JOHNSON. Preliminary inoculation experiments with *Fusarium* species on seedlings grown in nutrient agars. Trans. Ky. Acad. Sci. **2**: 111–117. 1927.
16. VORONIKHIN, N. N. (*Fusarium albizziae* nov. sp. as a possible cause of the destruction of the silkish acacia (*Albizzia julibrissin* Boiss.) in the Batum region. Prelim. rept.) Trans. title. Viestnik Tiflisskago Botanichiskago Sada. **48**: 15–36. 1920.
17. WELLMAN, F. L. A technique for study of pathogenicity or resistance in *Fusarium* wilt of tomato. Phytopath. **29**: 945–956. 1939.
18. ————, and D. J. BLAISDELL. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bull. 705, 28 pp. 1940.

FACTORS AFFECTING INFECTION IN RYE SMUT AND SUBSEQUENT DEVELOPMENT OF THE FUNGUS IN THE HOST[1]

LEE LING

(Accepted for publication November 12, 1940)

Previous work on *Urocystis occulta* (Waltr.) Rab., has shown that the moisture content and temperature of the soil are important in determining the extent of infection of rye by this smut (17). The effect of other factors, such as H-ion concentration of the soil, fertilizers, and light, has not been studied in detail. It was the writer's purpose in conducting this investigation to study various factors that influence the infection of rye and the later development of the smut within its host.

MATERIALS AND METHODS

Five commercial varieties of rye were used: Dakold (Minn. 93), Rosen (Minn. 82), Swedish (Minn. 2), the most commonly grown winter varieties, and the spring varieties Prolific (Minn. 89) and another sample (labeled KS-1) obtained from Northrup, King and Company, Minneapolis, Minnesota. The latter will be designated hereafter simply as spring rye. The smut used throughout the work was collected at University Farm, St. Paul, Minnesota.

Rye seeds were inoculated either by dusting heavily with dry chlamydospores or by soaking in a heavy spore suspension for a day. The latter method was more satisfactory and was used in certain experiments in the greenhouse. For field experiments, 1.5 to 2 g. of inoculum was used for each 100 g. of rye seed.

In greenhouse experiments, 6-in. flower pots, at least in quadruplicate, filled with steamed soil composed of two parts loam and one part sand were used, except in the study of soil moisture and soil nutrition in which glazed jars were used. Infected plants usually were not counted until 3 months after planting, which insured the maximum development of smut. Because of the dense stand in field studies, culms were counted instead of plants. Additional materials and methods will be described whenever necessary.

SOIL FACTORS

The effect of soil temperature and soil moisture on infection of rye by *Urocystis occulta* was reported by Stakman, Moore, and Cassell (17), and later by Ling and Moore (13). They found that infection occurred at temperatures ranging from 5 to 25° C. The optimum was between 13 and

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[1] A portion of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy. Degree granted by the University of Minnesota in June, 1937.

The writer wishes to thank the several people who encouraged him and criticized the work during its progress: Dr. E. C. Stakman, Dr. J. J. Christensen, Dr. Ian W. Tervet, and Mr. M. B. Moore.

17° C., varying with the depth of planting. High soil moisture markedly reduced the percentage of smutted plants and also narrowed the thermal range for infection. The following experiments were a continuation of the work mentioned above, and included a study of the effects of several additional environmental factors on infection and development of *U. occulta.*

Hydrogen-ion Concentration

To obtain a range in hydrogen-ion concentrations, hydrochloric acid and hydrated lime were added to a series of soils. Hydrated lime was thoroughly mixed with the soil at the rate of from 3 to 30 g. per kg. of soil; and hydrochloric acid was applied in normal solution at 10 to 50 cc. per kg. of soil. pH was measured electrometrically by the Division of Soils, University Farm, St. Paul, Minnesota. The pH of untreated soil was 7.2. Two hundred inoculated seeds of Dakold rye were planted in each of the soils in flats. The seedlings were counted 9 days after planting and then transplanted into the nontreated soil in pots, where they remained until notes were taken. Thus only seed germination, development of seedlings, and infection by the fungus were influenced by the hydrogen-ion concentrations of the soil, while later development was under uniform conditions in the greenhouse. The results (Table 1) indicate that the percentage of emergence of rye seedlings apparently was not affected by the hydrogen-ion concentration and that infection reached the maximum at pH 7.36, beyond which it decreased. The plant vigor at the end of the experiment also was in no way affected by the treatments in the seedling stage.

TABLE 1.—*Effect of soil reaction on infection of Dakold rye by Urocystis occulta*

Soil pH	Number seedlings emerged[a]	Number plants after three months	Per cent plants infected
5.49	140	122	35
5.87	134	118	32
6.36	138	114	40
7.36	141	117	50
8.58	132	122	34
8.92	145	138	17
10.14	140	134	4
11.10	146	139	3
11.92	143	138	1

[a] 200 seeds were planted in each series.

Moisture

From results of many investigators, it is well established that soil moisture has a decided effect on infection by cereal smuts. Reduction in infection may take place toward either extreme of soil moisture. According to Jones (8), and Bartholomew and Jones (2), high soil moisture inhibits both the spore germination of *Ustilago avenae* (Pers.) Jens. and the percentage of infection in oats. There is no evidence, however, regarding the effect of soil moisture on the development of smut in the host after infection has occurred. Experiments therefore were made in the greenhouse in an attempt to determine the extent of this influence.

In each of the two experiments made, 18 glazed ½-gal. crocks were filled with equal amounts of air-dried soil, the water-holding capacity of which had been determined previously. The bottoms of the crocks were covered with a layer of pebbles to facilitate watering. Inoculated seeds of Rosen rye were planted and the soil was kept at approximately 25 per cent of its water-holding capacity in all the crocks. Ten days after planting, the crocks were divided into 3 groups of 6 each. Soil in one group was retained at 25 per cent water-holding capacity, and sufficient water was added to the crocks in the other two groups to bring the soil moisture to 50 and 75 per cent, respectively. The water loss from transpiration and evaporation was determined by weighing the crocks daily and comparing with the original weight; it was replenished by means of a glass tube extending through the soil to the bottom of the crock.

The results (Table 2) show that the development of *Urocystis occulta* in the host, subsequent to infection, like the initial infection reported by Ling and Moore (13), was favored by a relatively dry soil. As the pathogen was no longer affected directly by soil conditions after its entrance into the host, the effect on the fungus must have been indirect, through some change in the vital processes in the host cells.

TABLE 2.—*Effect of soil moisture on development of Urocystis occulta in Rosen rye*

Soil moisture content, in percentage[a]	First experiment		Second experiment	
	Total number plants	Per cent plants infected	Total number plants	Per cent plants infected
25	63	35	81	26
50	61	29	82	24
75	51	14	80	19

[a] Based on water-holding capacity of soil and determined on air-dry basis.

Nutrition

Experiments were made to determine the influence of fertilizers on the development of rye smut. The tests were conducted at Coon Creek, Minnesota, on a very poor sandy soil, using sodium nitrate, treble superphosphate, and potash at 200, 200, and 400 pounds an acre, respectively, alone and in combination. Very little smut developed (about 1 per cent); however, no definite conclusions can be drawn from the data. Later, similar experiments were made in the greenhouse with ordinary sand, using the same fertilizers. There were no significant differences in the percentage of smut, which ranged from 14 to 24 per cent, as a result of those treatments.

Sand Culture in Greenhouse

Since it was impossible definitely to control the nutrients in soil, another experiment was made by growing rye plants in 3-salt nutrient solutions in sand culture. Inoculated seeds of Dakold rye were germinated on wet filter paper in large Petri dishes at 15° C. After 5 days, the seedlings were transplanted into quartz sand in glazed crocks 12 cm. deep and 16 cm. in diameter. Seedlings were selected at random without consideration for deformities, as these might have resulted from smut infection. The sand was of medium fineness, with a water-holding capacity of 32 per cent on the dry-weight basis. Seven culture solutions were selected from those described by Trelease and Trelease (19), to represent a wide variation in salt proportions. Each had a total concentration of 0.02 g. mol. per l. In addition to the 3 main salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ each solution contained 0.000001 g. molecule of $FeSO_4$ per l. The solutions were renewed every week. For supplying and removing the solution, the method described by Livingston (14) was followed. The moisture content of sand was maintained approximately at 50 per cent of water-holding capacity.

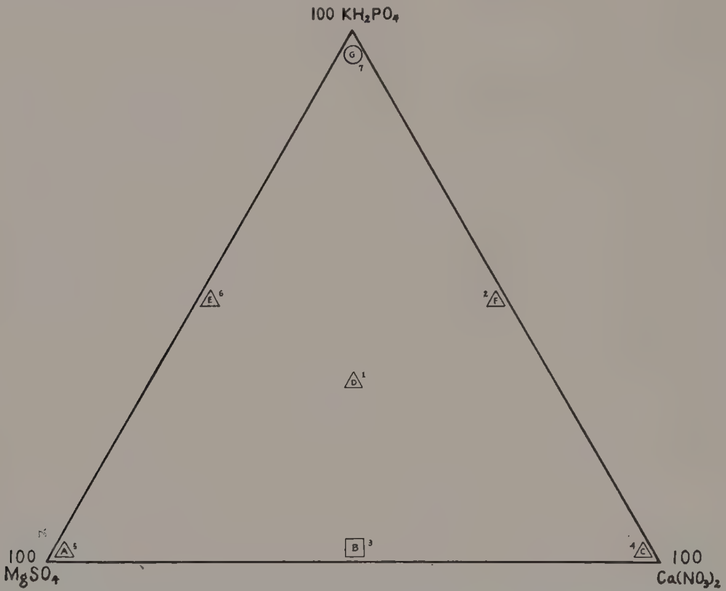

FIG. 1. Relative dry weight of Dakold rye and degrees of infection by *Urocystis occulta.* Letters designate the corresponding solutions. Numbers represent relative dry weights. Low, medium, and high infections are indicated by circle, triangles, and square, respectively.

Table 3 gives the volume-molecular proportions of the 3 salts, together with the dry weight of healthy and infected plants and the percentage of smut infection. The same data are plotted in the triangular diagram of figure 1. The best growth occurred in solution D, which had equal molecular proportions of the 3 main salts. $Ca(NO_3)_2$ was apparently more influential in determining the dry weight than either of the other salts. From the diagram it is obvious that the area along the left edge of the triangle is occupied by 3 solutions where the growth was poor and represents the lowest partial concentration of $Ca(NO_3)_2$. The dying of basal leaves, however,

TABLE 3.—*Effect of salt nutrition on dry weight of Dakold rye and its infection by Urocystis occulta in sand culture*

Solution	Molecular proportions[a]			Average dry weight of tops per plant, in mg.		Total number plants	Number plants infected	Per cent plants infected[b]
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Healthy	Infected			
A	2	2	96	97	71	85	16	19
B	2	49	49	334	271	77	28	36
C	2	96	2	245	225	84	16	19
D	33.3	33.3	33.3	506	399	82	20	24
E	49	2	49	67	47	85	18	21
F	49	49	2	391	372	85	18	21
G	96	2	2	63	50	81	4	5

[a] The molecular proportions represent percentages of 0.02 gram-molecule per liter.
[b] Minimum mean difference requirement for significance is 13.3.

appeared to be more severe in these 3 solutions, as well as in solution D. The dry weight of infected plants was consistently lower than that of healthy plants, being 71 to 95 per cent of the latter.

Regarding the smut infection, it is shown in table 4 that the variance due to nutrient solutions is significant. The minimum mean difference requires a value of 13.3 for significance. Thus the infection in solution B was significantly higher than in others, with the exception of solution D; and it was significantly lower in solution G. In the triangular diagram, if a line were drawn from the upper apex to the middle of the bottom edge, the high and low infections would lie on two extremes of the line, respectively, while medium infections occurred in the middle of the line and also in the area beyond the line. This line would represent the partial concentrations of KH_2PO_4, decreasing toward the lower end. Such a relation also appeared to be true in the case of susceptibility of wheat to mildew, as studied by Trelease and Trelease (19).

TABLE 4.—*Analysis of variance of smut infection in Dakold rye in sand culture*

Variance due to	Degrees of freedom	Sum of square	Variance	F
Replications	3	1,215.07	....	....
Solutions	6	2,083.24	347.21	3.90[a]
Error	18	1,603.23	89.07	....
Total	27	4,901.54		

[a] Exceeds 5 per cent point, but fails to reach 1 per cent point.

The results suggest that the amount of smut infection in this experiment was chiefly determined by molecular ratios of KH_2PO_4 to $Ca(NO_3)_2$ and $MgSO_4$ in the solution. Apparently, there is no correlation between host vigor, as indicated by dry weight of uninfected plants, and susceptibility to smut.

LIGHT FACTORS

Photoperiodism

Since the extensive investigations of Garner and Allard, a considerable amount of research has been done on the effect of the relative length of day and night upon growth and reproduction in plants. Leukel (10) reported a slight increase in the percentage of loose-smut infection in barley plants receiving a normal period of daylight over that of those exposed to additional artificial illumination. To determine the effect of light period on interdependent development of rye and *Urocystis occulta* and also to find out whether such effect is sufficient to cause the variation between experiments in the greenhouse made at different times of the year, a spring variety, Prolific, and a winter variety, Dakold, were used. Different day-length conditions were provided as soon as the seedlings began to emerge. For the long-day plants, the natural day was supplemented from 11 p.m. to 7 a.m. every day with light from 500-watt Mazda lamps in deep-bowl reflectors. The intensity of artificial light at the level of soil was about 200 foot-candles. Under artificial illumination, air temperature at the level of soil was raised approximately 1.5° C., but there was no appreciable change in soil temperature. The short-day condition was produced for the plants by means of a frame covered with 2 layers of heavy brown wrapping paper. The plants were uncovered in the middle of the day to receive a light period from 6 to 8 hours. Control plants with the natural light period were protected from the artificial light by a curtain. The natural day length increased over the period of experiment from 9 to 11 hours.

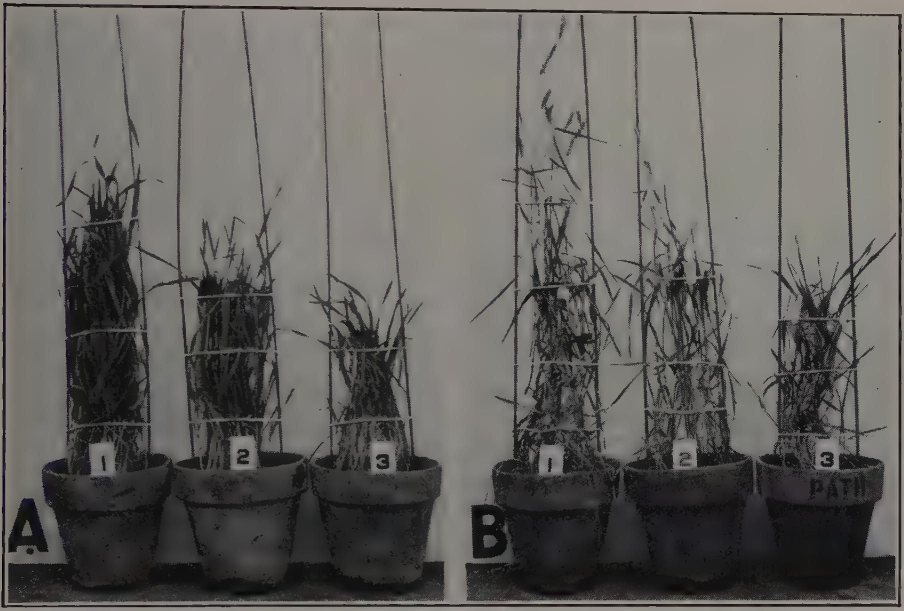

FIG. 2. Effect of length of day on growth of Dakold rye (A) and spring rye (B). 1, long day (17–18.5 hours); 2, natural day (9–11 hours); 3, short day (6–8 hours).

In agreement with the observation of Adams (1), the rate of growth was more rapid at first in the rye seedlings grown in a short-day condition, but later those plants exposed to long day attained greater height (Fig. 2). As shown in table 5, the dry weight of healthy plants of Dakold increased with increase in the length of exposure to light. In the case of Prolific, however, the short-day condition produced more dry matter in plants than the natural one, by increasing the number of tillers. The infected plants were more stunted and significantly reduced in dry weight. Dakold, the winter rye, failed to head at the end of the experiment, probably because of the rather high temperature (17–20° C.) at the time of germination. Purvis (16) also mentioned that day length and the temperature of germination are two interdependent factors determining flowering in winter rye. Prolific, the spring variety, produced normal heads under the exposure to long and to natural light periods and only a few small heads in the short-day series.

TABLE 5.—*Effect of day length on development of Urocystis occulta in spring (Prolific) and winter (Dakold) rye*[a]

Day length	Prolific[b]							Dakold[c]				
	Total number plants	Dry weight of tops per plant, in mg.		Date of first heading	Number plants headed	Date of first appearance of smut	Per cent plants infected	Total number plants	Dry weight of tops per plant, in mg.		Date of first appearance of smut	Per cent plants infected
		Healthy	Infected						Healthy	Infected		
Long (17–18.5 hrs.)	72	334	192	Jan. 26	55	Jan. 10	28	63	462	244	Jan. 2	48
Natural (9–11 hrs.)	63	212	168	Feb. 3	33	Jan. 21	30	51	335	252	Jan. 11	39
Short (6–8 hrs.[d])	40	259	178	Feb. 16	6	Jan. 27	27	63	236	117	Jan. 22	29

[a] Notes taken March 2, 1937.

[b] Planted December 12, 1936.

[c] Planted December 4, 1936.

[d] Plants in the short-day series, received only 6 hours of light each day at the first month after planting. After the first month, those plants were exposed to natural day length for a week, since they appeared very weak and hardly continued to grow. Later, the day length for this series was increased to 8 hours each day.

The development of the fungus in both varieties also was retarded by the short-day condition and hastened by the long day. The final percentage of infection, however, was increased only in Dakold with the lengthening of the light period, but it was not significantly affected in Prolific. Additional experiments were made in the greenhouse to test the effect of light intensity on development of *Urocystis occulta* in Rosen rye. In plants exposed to reduced light intensity the first perceptible smut lesions were always observed 2 to 4 days later than in those receiving full daylight, but the final amount of infection was very similar in the two groups.

RELATION OF SEEDLING VIGOR TO SMUT INFECTION

Rate of Seed Germination

Since the time of Brefeld, many investigators have endeavored to interpret the variation in smut infection under diverse conditions by differences in the rate of germination and subsequent growth of the seedlings of the host. Attempts even have been made to correlate the varietal resistance with the speed of germination, though accumulated evidence indicates that the complex nature of disease resistance can hardly be explained in so simple a manner. However, within a variety, the rapidity of seed germination possibly has some influence on the amount of infection. This has been shown for bunt-infected wheat by Faris (4), who found that more infection occurred in the wheat seedlings, which were quicker to emerge from the soil. The present investigation was undertaken with both *Urocystis occulta* and *Tilletia levis* in order to have a direct comparison with Faris' results.

Seed of Dakold rye and Ceres wheat was inoculated with *Urocystis occulta* and *Tilletia levis*,[2] respectively, and planted in pots at a uniform depth of one inch. The pots were kept in the greenhouse at different temperatures during the first two weeks after the seeds were sown. In each temperature series plants were divided into 3 groups. In one group, only the seedlings which had emerged in the first 2 days after the appearance of the first plant were retained, and all those appearing later were removed. In another group, only the late seedlings were permitted to grow. The check consisted of all seedlings, none being removed. The results (Table 6) confirm the work of Faris. However, only under lower temperatures, *i.e.*, 15° C. for *Urocystis occulta* and 10° C. for *Tilletia levis*, is it true that the more rapidly emerging seedlings had a higher percentage of smut than those of slower growth. The amount of smut in check plants was intermediate between the two. At higher temperatures, however, there was no such difference. Faris explained his results on the basis of the difference in host vigor, more vigorous seedlings being more subject to infection. In reality, however, late seedlings of wheat and rye were not necesarily very much weaker than those emerging first.

[2] The material was collected by Mr. W. Butler in the state of Coahuila, Mexico, April, 1936.

TABLE 6.—*Effect of rapidity of germination of rye and wheat seed on the infection of Dakold rye by Urocystis occulta and Ceres wheat by Tilletia levis*

Temperature of germination, in °C.	Time of seedling emergence	Total number of plants or heads	Per cent plants or heads infected
	Urocystis occulta		
15	Early	124	46
15	Late	65	37
15	Check	129	39
20	Early	164	27
20	Late	53	28
20	Check	121	26
	Tilletia levis		
10	Early	160	61
10	Late	93	39
10	Check	159	56
15	Early	50	14
15	Late	42	14
15	Check	73	23

Date of Seeding

In 1935 and 1936 rye was sown on various dates in the spring and again in the fall, each over a period of a month or more, to determine the influence of date of planting on the incidence of smut. Figure 3 gives, for the 1936 fall planting, a graphic summary of the relation of soil temperature, soil moisture, rainfall, and date of planting to the amount of rye smut.

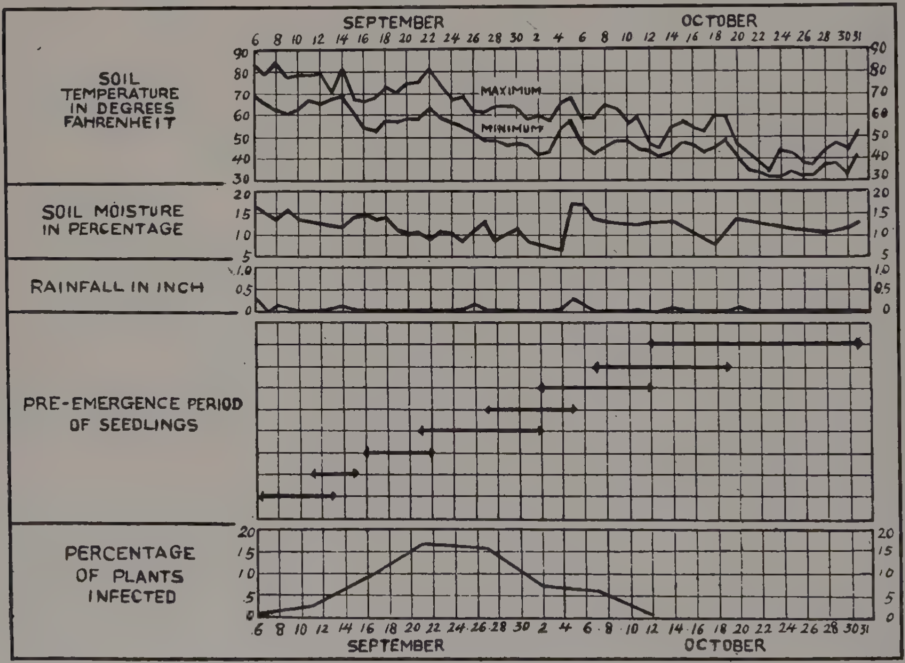

FIG. 3. Graphic representation of factors affecting the infection of Dakold rye by *Urocystis occulta* at different dates of sowing.

For spring rye, the maximum percentage of infection (3 per cent) was produced on the April 20 planting, which is about the usual time for seeding rye in central Minnesota. There was a trace of smut in later sowings until May 19, after which no infection was found. The soil temperature fluctuated considerably throughout the whole planting period from a minimum of 37.6 to 61.7° F. It was not too high for the germination of chlamydospores, even at the time of the last seeding, while the distribution of rainfall was fairly even. The elimination of smut was probably caused by the rapid rise in temperature during late spring, the high temperature accelerating the growth of plants, which probably escaped infection to a great extent for this region. In 1935–36 most smut was found on the first two plantings, 9 per cent occurring on the planting of September 21 and 11 per cent on that of September 26. For the succeeding year, again most smut occurred on plants sown on September 21, 17 per cent; and September 27, 15 per cent. In 1935 and 1936, the soil temperature during the pre-emergence period of seedlings planted between September 21 and 27 remained close to 58° F. the optimum for infection. In October, the temperature dropped, thus preventing to a considerable extent the germination of chlamydospores. The rainfall throughout September and October was low, and the soil moisture did not seem to be an important factor for infection, as moisture sufficient for germination of rye seeds would also be sufficient for germination of chlamydospores. Several factors relating to smut infection during the period rye was planted in 1936 are presented in figure 3.

Depth of Seeding

Woolman and Humphrey (22) showed that there was a direct correlation between the depth of seeding and the percentage of bunt infection in wheat. Taylor and Zehner (18) also reported a similar case in susceptible varieties of winter barley infected by *Ustilago hordei* (Pers.) Kell. and Sw. and *U. nuda* (Jens.) Kell. and Sw. If the susceptible stage of seedlings were limited to the period of pre-emergence, the liability to infection would be increased by deep sowing. Field data obtained from 1935 to 1937 with both spring and winter rye show that such a tendency also existed in plants infected by *Urocystis occulta,* but less definitely. It was found that with Dakold rye in 1935–36, seeds planted at a depth of ½ inch had 5 per cent smut, at 1 inch 8 per cent, at 1½ inches 14 per cent, and at 2 inches 11 per cent. The data for the years 1936–37, are as follows: ½ inch depth 6 per cent, 1 inch 15 per cent, 1½ inches 13 per cent, and 2 inches 14 per cent. On the basis of these results it may be concluded that shallow planting, ½ inch, decreases the amount of smut, while in plantings made from 1 to 2 inches more smut is produced, although the amount of infection did not increase uniformly with increasing depth of seeding. Data from spring rye were inconclusive. It was rather difficult to obtain a uniform depth of seeding in the field.

EFFECT OF UROCYSTIS OCCULTA ON WINTER SURVIVAL OF RYE

The ordinary occurrence of a fairly low percentage of smut in winter rye has suggested that most infected plants are predisposed to winter killing. Following the severe winter of 1935–36, field observations showed that, at least in the variety Dakold, more smutted plants were killed than smut-free ones. In 1936–37, experiments were made with 3 winter varieties, each inoculated with 3 different amounts of smut. The seed was space-planted. The plants were counted soon after emergence and again at maturity, the percentage survival being computed on this basis. The results are presented in table 7.

TABLE 7.—*Effect of infection by Urocystis occulta on winter survival of rye*[a]

Variety	Grams of inoculum per 100 g. seed	Number seedlings emerged	Stand at maturity	Per cent plants survived	Per cent plants infected
Dakold	2.5	134	105	78	23
	1.5	100	81	81	26
	0.5	147	133	90	18
	check	139	128	92	0
Rosen	2.5	154	123	80	12
	1.5	136	110	81	6
	0.5	147	122	83	6
	check	158	128	81	0
Swedish	2.5	101	79	78	29
	1.5	112	96	86	18
	0.5	105	92	88	18
	check	108	90	83	0

[a] Results are based on triplicate rows, each sown with 100 seeds.

In comparing the rate of germination of inoculated seeds and check there was no indication that smut affects germination of the seed. The winter was rather mild for a conclusive test of the hardiness of rye. Only in Dakold, the most winter-hardy variety grown, was there any indication that smut-infected plants were predisposed to winter injury in this region. In this case 92 per cent of the plants survived in the check as compared with 78 per cent in the most heavily inoculated plots. The other two varieties, Rosen and Swedish, are more susceptible to winter injury, but failed to show any appreciable difference in percentage survival between infected and noninfected plants.

CROSS INOCULATION ON WHEAT

The fungus causing flag smut of wheat was first determined by Wolff (21) as *Urocystis occulta.* Koernicke (9) separated *U. tritici* as a distinct species on the basis of morphological characters alone. McAlpine (15) concluded from his experiments of cross inoculation with *Urocystis* from wheat and rye that the two species were parasitically distinct. His results later were confirmed by Griffiths (6) in the United States and by Verwoerd (20) in South Africa. The writer's data also agree with McAlpine. Negative results were obtained when 5 varieties of winter wheat and 4 of spring wheat were inoculated with *U. occulta*. The following varieties were tested: Harvest Queen (source unknown), Fultz (C.I. 1923), Jones Fife (C.I. 6177), Red May (C.I. 5620), Red Rock (C.I. 6951), Mindum (C.I. 5296), Kota (C.I. 5878), Kubanka (C.I. 2094), and Acme (C.I. 5284). Rosen and Dakold rye had 24 and 38 per cent infection, respectively. *Urocystis tritici* was not included, as it does not occur in this region.

CONTROL MEASURES

Seed Treatment

During 1935 and 1936 a number of commercial fungicides were tested for their effectiveness in controlling the flag smut of rye. The amount recommended by the manufacturers was applied. Most of the fungicides gave excellent control, only a trace of smut occurring in the majority of the treated plots of winter rye, while in the control plants 15 per cent smut was found in 1935–36 and 12 per cent in 1936–37. So little smut appeared in the control plots of spring rye in 1935, that information regarding the efficacy of seed treatment could not be obtained. Only 2 treatments gave more than a trace of smut in the 2 years' testing of Dakold rye. With 20 per cent copper carbonate, 3 per cent smut occurred in 1935–36, while formaldehyde dip allowed 2 per cent in 1936–37. In both 1935 and 1936 the period following sowing was very dry and considerable reduction in stand was noticed in seeds treated with formaldehyde dip and Corona-formaldehyde dust. Particularly during 1936, both treatments also retarded the germination of seeds 2 to 3 days later than the check, and caused appreciable stunting in the seedlings. With the two organic mercury dusts, Ceresan and New Improved Ceresan, the stand was increased, probably due to the protective action of these fungicides against the soil-borne pathogens.

The Effect of Picric Acid Added to Soil

Recently evidence has been obtained to show that resistance of wheat and barley to *Puccinia triticina* Eriks. and *P. simplex* (Koern.) Eriks. and Henn. could be induced by applying organic compounds to soil (5, 7). Picric acid, one of the compounds used by these workers, was tested for the possibility of chemical immunization of Dakold rye attacked by *Urocystis occulta*. Picric acid was applied at various times at rates of 60 to 240 mg. per kg. of soil. It was applied after the emergence of seedlings in one or several applications, to prevent its direct influence on germination of chlamydospores. The smallest amount of picric acid, 60 mg. per kg. of soil, applied once, gave 34 per cent smut, while one application of 120 mg. gave 31 per cent. One hundred twenty mg. picric acid applied in 4 doses at 3-day intervals reduced the percentage of smut to 22, while 240 mg. of dust applied in 4 doses at 3-day intervals gave 20 per cent. The untreated plants had 37 per cent smut. Several applications of acid at intervals seemed to be more effective than a single one. While the amount of smut was reduced, there was some injury to the plants, the injury increasing with the higher concentrations of acid.

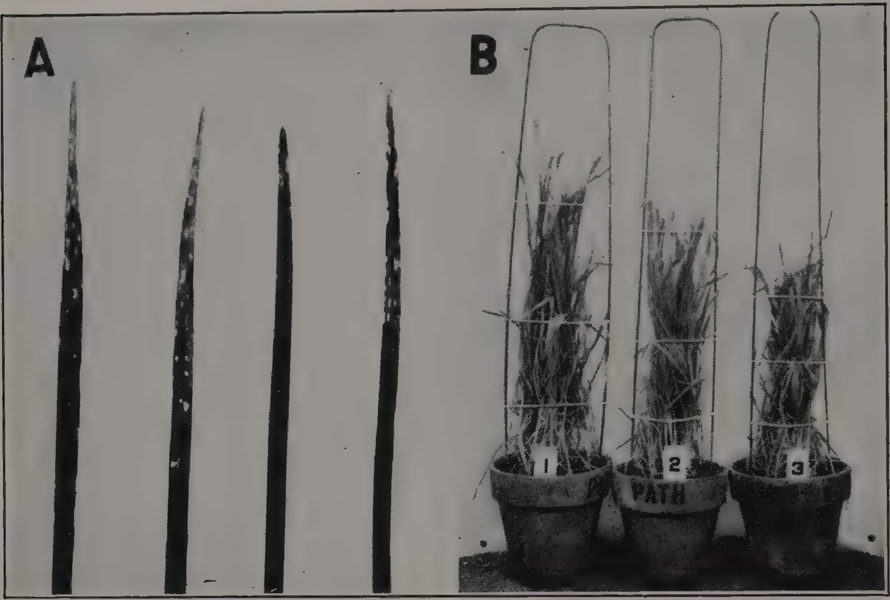

FIG. 4. A. Necrotic spots developed on leaves of Dakold rye treated with picric acid. B. Stimulative and stunting effects of picric acid on growth of Dakold rye. 1, Treated with 60 mg. of acid per kilogram of soil; 2, check; 3, treated with 240 mg. of acid per kilogram of soil.

The damage caused by picric acid on Dakold rye usually was more severe than that on wheat. About 5 days after applying the acid, rye seedlings developed necrotic spots, and the tips of the leaves began to wither (Fig. 4, A). The necrosis persisted on mature plants to the end of the experiment. The growth of plants, however, was stunted only when they were treated with a heavy dose of picric acid, while a light dose apparently had a stimulating effect (Fig. 4, B). The data obtained indicate that the degree of injury was increased and the percentage of infection was decreased with an increase in the dose of picric acid, but it is not suggested that picric acid treatment of the soil would be practicable for controlling rye smut.

DISCUSSION

The establishment of parasitism is dependent on a fine adjustment in the host-pathogen complex. Consequently, external factors may influence such an adjustment in the following ways: (1) by altering the susceptibility of the host; (2) by altering the aggressiveness of the pathogen; (3) by a combination of both. Sometimes, however, it is rather difficult to evaluate the relative importance of these effects. In analyzing such complex conditions, emphasis must also be laid upon the time factor involved, for the length of time during which the pathogen acts upon the host is always a matter of importance. As suggested by Brefeld (3), environmental factors may affect the relative growth of host and pathogen in a differential way, and once the harmonious relationship between the two organisms becomes disturbed, the parasitism may no longer be maintained. This may occur any time during the incubation period, or even before the entrance of the pathogen.

The rapidity of germination of chlamydospores of *Urocystis occulta* is influenced profoundly by temperature and also, to a lesser extent, by the substratum, although the total germination may be similar in all instances (11). The significance of the speed of germination may easily be overlooked, but, in nature, this is of great importance. It has been shown that rye seedlings are susceptible only until the emergence of the first true leaf, and thus infection necessitates germination of the spores within a certain time. In this case, the soil temperature during the time of seed germination must be a limiting factor in the establishment of the disease. High temperatures may hasten the germination of the rye seeds but delay the germination of the chlamydospores, or a low temperature may retard the germination of the fungus more than that of the host, so that the pathogen will miss the opportunity of entering the host.

Having gained entrance, the ability of the pathogen to establish itself will depend upon its reaching the meristematic region of the host within a limited length of time and keeping pace with it (12). Photoperiodism affects the development of the two organisms in a parallel way, for, especially in Prolific rye, the appearance of smut sori occurred consistently from 13 to 20 days before the time of heading.

Many investigators have emphasized the relation between host vigor and the development of disease. In the present study, however, there was no indication of correlation between the two. Light is one of the important factors influencing plant vigor. In Dakold rye, the percentage of smut was increased with an increase in light exposure, although this may be more reasonably attributed to factors other than host vigor, but there was no difference in the amount of smut infection in Prolific and Rosen rye grown under various light conditions. Likewise, the degree of vegetative vigor of rye in sand cultures, as influenced by salts supplied to the roots, was not correlated with the degree of susceptibility to smut. The most vigorous plants were moderately susceptible; less vigorous ones moderately to highly susceptible; while least vigorous ones were least to moderately susceptible.

There are many contradictions in the literature regarding the effect of certain nutrient elements on the susceptibility of plants to smut. Most of these investigations, however, were made in ordinary field or greenhouse soil, the composition of which was not controlled in the field or in the greenhouse, and in which the variation probably would be the cause of divergent results. In the present study, results obtained with sand cultures indicated that a solution high in KH_2PO_4 and low in $Ca(NO_3)_2$ and $MgSO_4$ decreased the number of smutted plants while a relatively high concentration of

$Ca(NO_3)_2$ and $MgSO_4$ with low concentrations of KH_2PO_4 increase the number of smutted plants. The range in concentration of the 3 salts, which can be obtained in culture solutions cannot be duplicated in soil. However, no attempt was made to differentiate between the action of phosphorus and potassium.

Under natural conditions, hydrogen-ion concentration does not seem to be a limiting factor in the occurrence of the disease. The development of the pathogen, including the germination of chlamydospores, infection, and growth on artificial media, can take place over a wide range of pH. Spore germination and infection of seedlings have a similar range of pH, the optima being 6.86 and 7.36, respectively. However, as the actual pH values in the two separate experiments did not correspond exactly, they have possibly a true optimum, lying between pH 6.86 and pH 7.36. The hydrogen-ion concentration of the soil may, therefore, determine infection through its effect on spore germination rather than through any influence on host resistance. For the growth of the fungus on artificial medium, the optimum pH is lower than that for spore germination. In Dakold rye, the acidity of expressed sap also undergoes such a change, becoming more acid toward the mature stage. Samples of plants 1 week old had an average pH of 6.16, while in those 12 weeks old it was 5.91. Whether such coincident change has any significance in the development of the pathogen in the host is hard to determine.

SUMMARY

A study was made of factors affecting infection by and subsequent development of *Urocystis occulta* on rye.

It was shown that the greatest amount of smut was produced on rye when inoculated seeds were allowed to germinate in a soil at pH 7.36.

Further, a relatively dry soil not only favored infection but subsequent development of the fungus in the host.

Experiments with fertilizers carried out in a poor, sandy soil did not affect the amount of smut produced, but with sand cultures under greenhouse conditions, results suggest that solutions, high in potassium phosphate and low in calcium nitrate and magnesium sulphate, decreases the amount of smut, while a reversal in these ratios tends to increase the amount of smut.

Variation in the light source, in regard to intensity and length of exposure, had but little effect on the smut production in Prolific rye, while in Dakold lengthening of the light period increased the amount of smut somewhat.

Rapidity of germination of rye seed affected the amount of infection by *U. occulta,* for at low temperature (15° C.), but not at higher temperatures, seeds that germinated rapidly were more apt to be infected.

In Dakold and possibly other varieties, smut-infected plants tend to be more easily killed during winter than smut-free plants.

Picric acid at the rate of 240 mg. per kg. of soil reduced the amount of smut from 37 per cent to 20 per cent, while smaller amounts of picric acid

were less effective in reducing smut. However, the heavier applications of picric acid injured the plants.

LITERATURE CITED

1. ADAMS, J. Duration of light and growth. Ann. Bot. 38: 509–523. 1924.
2. BARTHOLOMEW, LUCILLE K., and EDITH SEYMOUR JONES. Relation of certain soil factors to the infection of oats by loose smut. Jour. Agr. Res. [U.S.] 24: 569–575. 1923.
3. BREFELD, OSCAR. Untersuchungen aus dem Gessamtgebiete der Mykologie. Heft 11. Die Brandpilze 2. 98 pp. (Münster). 1895.
4. FARIS, JAMES A. Factors influencing the infection of wheat by *Tilletia tritici* and *Tilletia laevis.* Mycologia 16: 259–282. 1924.
5. GASSNER, G., and K. HASSEBRAUCK. Untersuchungen zur Frage der Getreiderostbekämpfung mit chemischen Mitteln. Phytopath. Zeitschr. 9: 427–454. 1936.
6. GRIFFITHS, MARION G. Experiments with flag smut of wheat and the causal fungus, *Urocystis tritici* Kcke. Jour. Agr. Res. [U. S.] 27: 425–449. 1924.
7. HART, HELEN, and J. L. ALLISON. Toluene compounds to control plant disease. Phytopath. 29: 978–981. 1939.
8. JONES, EDITH SEYMOUR. Influence of temperature, moisture, and oxygen on spore germination of *Ustilago avenae.* Jour. Agr. Res. [U. S.] 24: 577–591. 1923.
9. KOERNICKE, F. Mykologische Beiträge. Hedwigia 16: 33–40. 1877.
10. LEUKEL, R. W. Factors affecting the development of loose smut in barley and its control by dust fungicides. U. S. D. A. Tech. Bull. 293. 1932.
11. LING, LEE. Factors affecting spore germination and growth of *Urocystis occulta* in culture. Phytopath. 30: 579–591. 1940.
12. ——————. The histology of infection of susceptible and resistant selfed lines of rye by the rye smut fungus, *Urocystis occulta.* Phytopath. 30: 926–935. 1940.
13. ——————, and M. B. MOORE. Influence of soil temperature and soil moisture on infection of stem smut of rye. Phytopath. 27: 633–636. 1937.
14. LIVINGSTON, BURTON E. A plan for cooperative research on the salt requirements of representative agricultural plants. Rept. Com. Div. Biol. Agr. Nat. Res. Council. 2nd ed. 1919.
15. MCALPINE, D. The smuts of Australia. 285 pp. (Melbourne). 1910.
16. PURVIS, OLIVE NORAH. An analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day. Ann. Bot. 48: 919–955. 1935.
17. STAKMAN, E. C., M. B. MOORE, and R. C. CASSELL. The pathogenicity and cytology of *Urocystis occulta.* (Abstract) Phytopath. 24: 18. 1934.
18. TAYLOR, J. W., and MARION GRIFFITHS ZEHNER. Effect of depth of seeding on the occurrence of covered and loose smuts in winter barley. Jour. Amer. Soc. Agron. 23: 132–141. 1931.
19. TRELEASE, S. F., and H. M. TRELEASE. Susceptibility of wheat to mildew as influenced by salt nutrition. Bull. Torrey Bot. Club 55: 41–67. 1928.
20. VERWOERD, LEN. The biology, parasitism and control of *Urocystis tritici* Koern., the causal organism of flag smut in wheat (*Triticum* spp.), and recording the occurrence of *Urocystis occulta* (Wallr.) Rab. in South Africa as the cause of "stem smut" in rye. Dept. of Agr., Union of South Africa Sci. Bull. 76. 1929.
21. WOLFF, REINHOLD. Beitrag zur Kenntniss der Ustilagineen. Bot. Zeitung 31: 657–661; 673–677; 689–694. 1873.
22. WOOLMAN, HORACE M., and HARRY B. HUMPHREY. Studies in the physiology and control of bunt, or stinking smut of wheat. U.S.D.A. Bull. 1239. 1924.

PARASITISM OF THE ROOT-KNOT NEMATODE IN LEAVES AND STEMS[1]

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Although nematodes of many species infest the aerial parts of plants, *Heterodera marioni* (Cornu) Goodey, 1932, the root-knot nematode, is known almost exclusively as a parasite of subterranean organs. This parasite's adaptability to varied nutrient conditions is evidenced by its host range, exceeding 1300 species (1), but the literature presents few reports of its infesting aerial plant parts. Taylor has observed that when Lima-bean stems are covered with soil, they may become infected (9, p. 139). Steiner, Buhrer, and Rhoads (7) have reported stem galls extending above the soil surface on *Thunbergia* spp., *Rheum rhaponticum,* and *Begonia* spp., on the first of which galls developed to enormous size. Recently, Steiner (6) has recorded severe injury to beans resulting from infection of stems, cotlyedons, and leaves of seedlings germinated in heavily infested soil. The writer has also observed galls on the hypocotyl of cowpea (*Vigna sinensis* Endl.) and okra (*Hibiscus esculentus* L.), and on both hypocotyl and cotyledons of tomato (*Lycopersicum esculentum* Mill.), resulting from entry of nematodes during germination.

The recent evidence that larvae of *Heterodera marioni* are attracted to excised pieces of leaves and stems (5), led to experimental infestations of the shoots of various plants. Typical galls and other pathological disturbances developed that are described and illustrated in this paper. These studies have helped also to elucidate several details of the parasitism of this nematode.

METHODS

Larvae of *Heterodera marioni,* essentially free from other species, were hatched from exposed egg masses on thoroughly washed roots of sugar cane (variety P.O.J. 2878) grown in naturally infested soil. These larvae were concentrated and washed by decanting and were freed from debris by passage through filter paper.[2]

Methods of infestation were chosen with regard to two considerations: first, that the larvae escape from droplets of water only with great difficulty and are unable to move from water into a substance even as soft as a weak agar jelly; and second, that they penetrate roots chiefly through immature tissues. With few exceptions, experimental infestations were, therefore, confined to buds or very young leaves, and the larvae were applied in a supporting medium. This usually was a finely pulverized, black, volcanic pumice, described elsewhere as black sand (5), selected because of its ease of wetting and its retentiveness of water. As a relatively nonabrasive medium, soil that had passed through a 150-mesh standard sieve was sometimes substituted. Plant parts to be infested were covered by adding alternately water and the dry medium, then a concentrated suspension of nematode larvae was added, followed by more dry medium to absorb excess water. Infested plants were held in a moist chamber usually for 48 hours, the supporting medium was then washed off and the plants returned to the glasshouse. Check plants were handled similarly except that no nematodes were applied.

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[1] Published with the approval of the director as Technical Paper No. 134 of the Pineapple Experiment Station, University of Hawaii.

[2] The suspension of nematodes with debris is poured into a small, folded filter paper in a stemless funnel and is washed down into the point. The funnel is then lowered until the tip of the paper makes contact with water in a small container, and the funnel is capped with a Petri-dish half to avoid desiccation. Within 12 to 48 hours most of the nematodes wriggle through the paper into the water.

For the demonstration of nematodes in tissues without sectioning, the acetone, osmic-acid, clove-oil method of Godfrey (3) was chiefly employed. With mature leaves of certain species, however, sufficient treatment with acetone to remove chlorophyll prevented the nematodes from darkening with osmic acid. A slower but more selective removal of chlorophyll was obtained by a brief treatment in the hot 80 per cent acetone followed by soaking as long as necessary in 70 per cent alcohol.

INFESTATION OF BUDS

Miscellaneous Plants

Infestation of vegetative buds of members of several families gave variable results. No galls nor evidence of establishment of the parasite were observed on *Bixa orellana* L., *Brassaia actinophylla* F. von Mueller, *Crotalaria spectabilis* Roth, *Euphorbia hirta* L., *Erigeron albidus* (Willd.) Gray, *Raimannia odorata* (Jacq.) Sprague and Riley, and *Vernonia cinerea* (L.) Less. Necrosis of young leaves occurred on *Brassaia* and *Vernonia*, and entire infested growing points of *Raimannia* died. With none of these plants were experiments sufficiently extensive to prove *Heterodera marioni* unable to establish, but these species appear less susceptible to leaf and stem infection than several plants to be considered.

Aerial gall development, sometimes with associated pathological conditions, followed infestation of plants of 6 species representing 5 families. Of these, the Whippoorwill variety of cowpea was studied most extensively and will be considered last.

When *Heterodera marioni* larvae were applied in black sand to the apical bud of tomato, Globe variety, nematodes established and developed to maturity in stems, petioles, rachises, petiolules, midribs, and small veins in the laminae. Galls appeared within a week and enlarged progressively until some of them exceeded 2 cm. in diameter (Fig. 1), representing effects of parasitism of several generations of nematodes out of contact with soil. From large galls on leaf rachises, short-lived root tips and, more rarely, dwarfed foliar axes were formed. These large galls superficially resembled the bacterial crown gall disease, but attempts to isolate pathogenic bacteria failed consistently.

With the Perfection variety pea, *Pisum sativum* L., a few experimental infestations of apical buds resulted in only occasional infections of leaflets and stipules. Larvae apparently penetrated with difficulty and chiefly in groups. Insufficient host accommodation in gall formation, accompanied by tearing of the tissues, was followed by early deterioration of the infected parts, and leaflets processed for microscopic observation after 22 days showed the parasites alive but poorly developed.

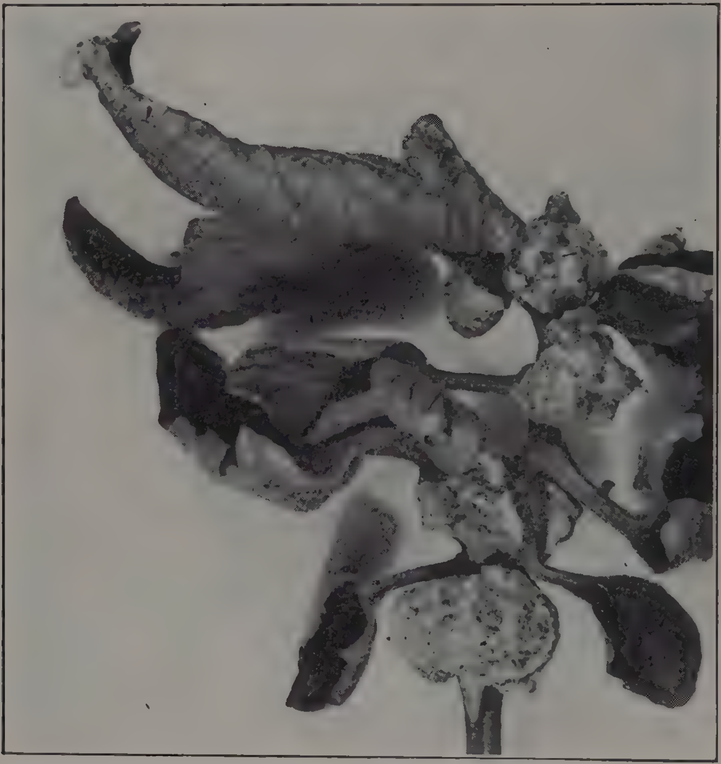

FIG. 1. Large compound galls on rachis and tip of terminal leaflet of mature tomato leaf infested 5 months earlier by applying larvae in black sand to the bud. Slightly reduced.

In leaf laminae and petioles of *Emilia sonchifolia* DC., numerous galls developed within which nematodes came to maturity and laid eggs. In laminae, the parasites were distributed on both large and small veins where they produced chiefly small, simple galls. On petioles, however, long compound galls soon developed rugose surfaces.

In the succulent leaves and stems of *Portulaca oleracea* L., the relatively few nematodes that established caused so little gall formation that when the first generation egg masses were well developed, infected parts were distinguished only with difficulty (Fig. 2, A).

Young pineapple (*Ananas comosus* (L.) Merrill) seedlings and the crowns of young inflorescences developed numerous inconspicuous leaf infections (Fig. 2, B). Giant-cell groups formed in the veins but remained small. The parasites developed slowly. Few of them matured, the first beginning to lay eggs after 2 months.

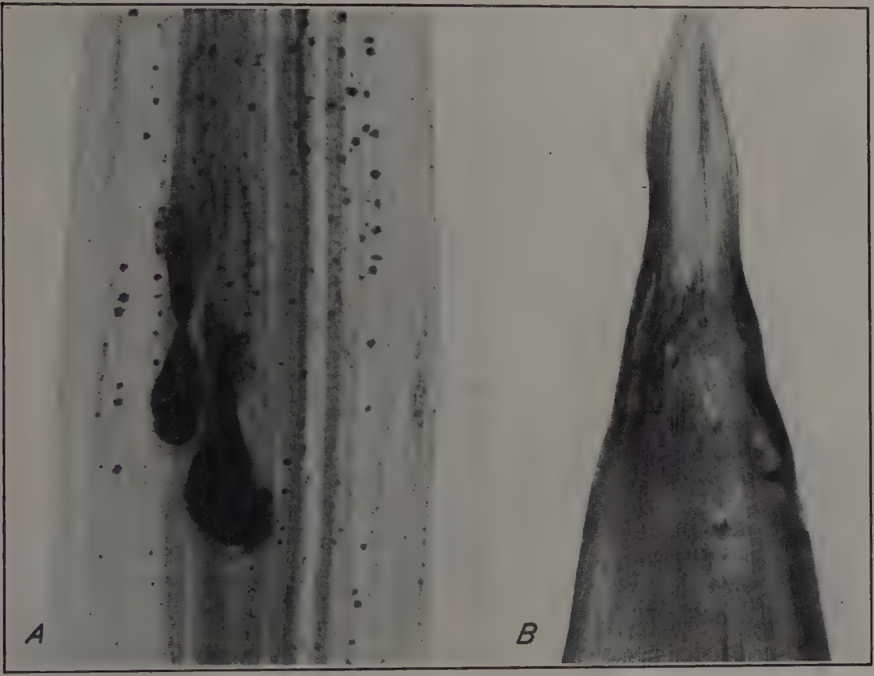

FIG. 2. A. *Heterodera marioni* females that have begun to lay eggs in very slightly hypertrophied stem of purslane. Heads are directed up the stem and giant cells extend up farther than down. ×24. B. Small galls on leaf of pineapple seedling. Few of the parasites matured to lay small numbers of eggs after 2 months. ×1.4.

Cowpea

Numerous experiments in which apical buds of Whippoorwill cowpea were infested immediately after separation of the first pair of simple leaves, or at later stages until the first trifoliate leaf was 5 mm. tall, proved the young leaves and stems of this plant to be very susceptible to the root-knot nematode. Localization of the parasites and the pathological disturbances that followed, varied with the stage of bud development and with the method of applying the infestation, whether with or without a supporting medium, but nearly every plant infested developed typical galls in which nematodes matured and laid eggs. Closely similar results were obtained with the Iron variety in limited trials, and the relatively nonabrasive soil proved as effective as black sand as a supporting medium, except for the greater difficulty attending its use. Somewhat fewer and generally lighter infections followed the application of a suspension of larvae alone.

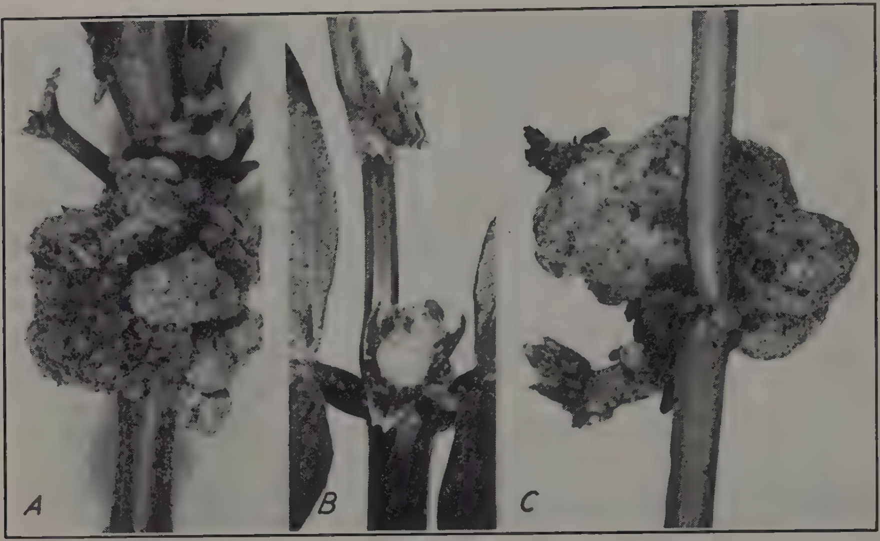

FIG. 3. Stem galls on Whippoorwill cowpea. A. Compound gall 5 months old on first internode, partly necrotic. Slightly reduced. B. Apical bud blinded but only slightly hypertrophied 24 days after infestation of the young bud. Notice the large abscission scar below the bud and the vigorous lateral shoot replacing the blinded bud. ×2. C. Terminal gall above first epicotyledonary node 3.5 months after infestation. A noninfected lateral shoot has replaced the terminal, and diminutive shoots extend from the gall surface. ×1.8.

Stem infections are of two classes. Some develop along the first epicotyledonary internode (Fig. 3, A), where a drop of infesting medium has been allowed to drain down from the bud during application of larvae. Fre-

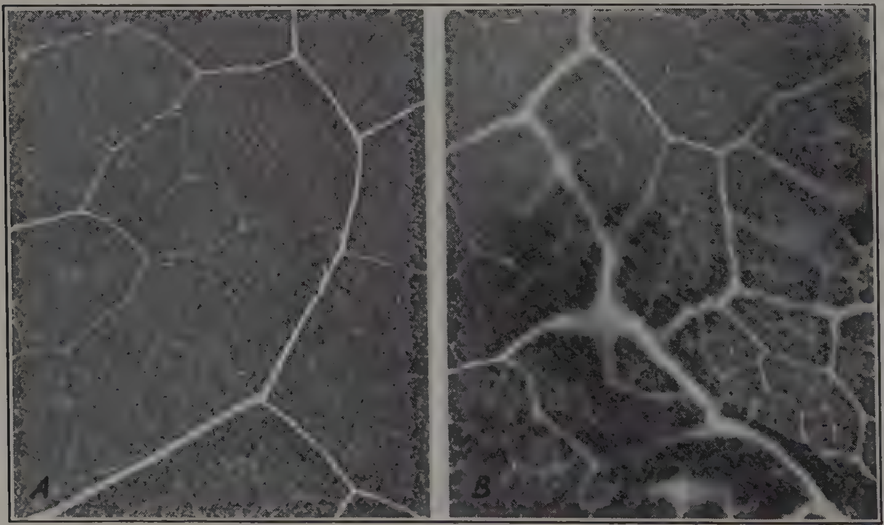

FIG. 4. Vein-clearing symptom of *Heterodera marioni* in Whippoorwill cowpea. A. Part of normal leaflet. B. Corresponding area of opposite leaflet of same leaf as A but heavily infected, showing vein clearing and galls. Both photographed with transmitted light. ×12.

quently, beginning as scattered simple galls, these enlarge and coalesce as succeeding generations of the parasites develop, becoming rugose galls of indeterminate size, sometimes resembling crown gall. Others develop at the stem apex when penetration has occurred at or near the apical primordium, blinding the shoot (Fig. 3, B and C, 6, A). From such galls, deformed lateral shoots sometimes arise, bearing diminutive leaves. This apical infection is most frequent following infestation of very young buds or infestation without a supporting medium. With soil or black sand on older buds, chiefly leaf infection is obtained.

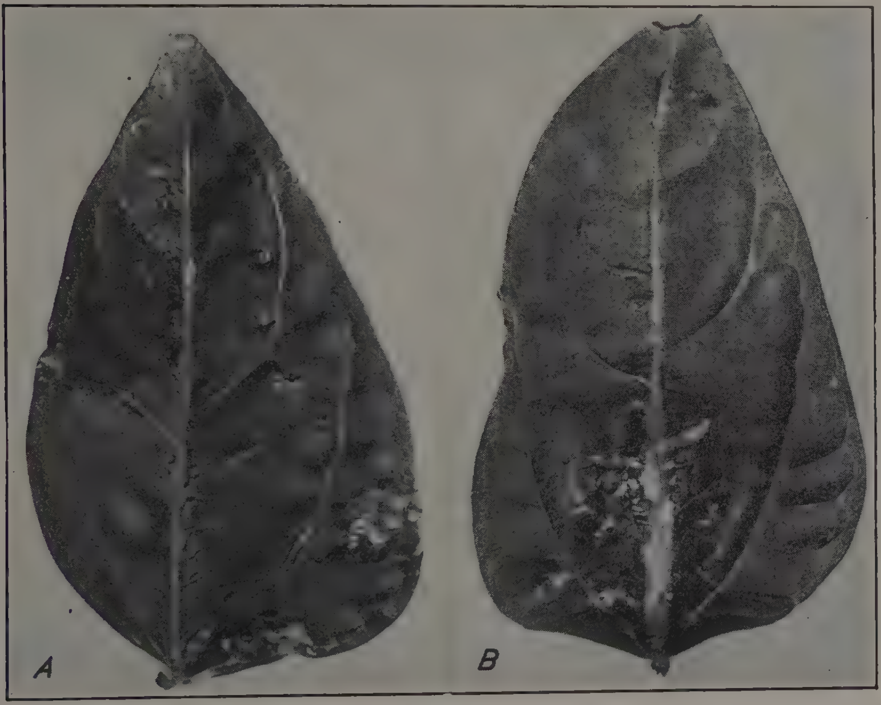

FIG. 5. Adaxial surfaces of Whippoorwill cowpea leaflets infested in the bud 40 days earlier with *H. marioni* larvae with black sand as a supporting medium. A. Heavy infection, well distributed, showing many galls of varied sizes. This leaflet contained 72 females, 26 males, and 18 immature or dead nematodes. See figures 8 and 9 for nematodes within this leaflet. B. Very heavy infection concentrated on or near midrib in basal half of leaflet, with a few galls more widely distributed.

Leaf infections were seen only in trifoliate leaves, the simple leaves being too old for establishment of the parasites when only half their mature length. First leaf symptoms, seen 48 hours after heavily infesting buds encased in a supporting medium, consist of an irregular and precocious unfolding of leaflets. Progressively, distortion becomes more evident, accompanied by mild mottling, vein clearing in the vicinity of developing parasites (Fig. 4), and occasional enations, giving some specimens a transitory resemblance to a virus disease. Galls become discernible within 5 or 6 days on midribs and more slowly on lateral veins (Fig. 5, A and B), and, as these enlarge and particularly as dark egg masses develop under the epidermis (Fig. 6, B), the nature of the infection becomes clearly apparent. In contrast with tomato leaves and cowpea stems, the new generation of larvae seems unable to feed in cowpea leaves. Heavily infected leaflets, particularly leaflets borne on heavily infected petioles, early become senescent (Fig. 6, A and B).

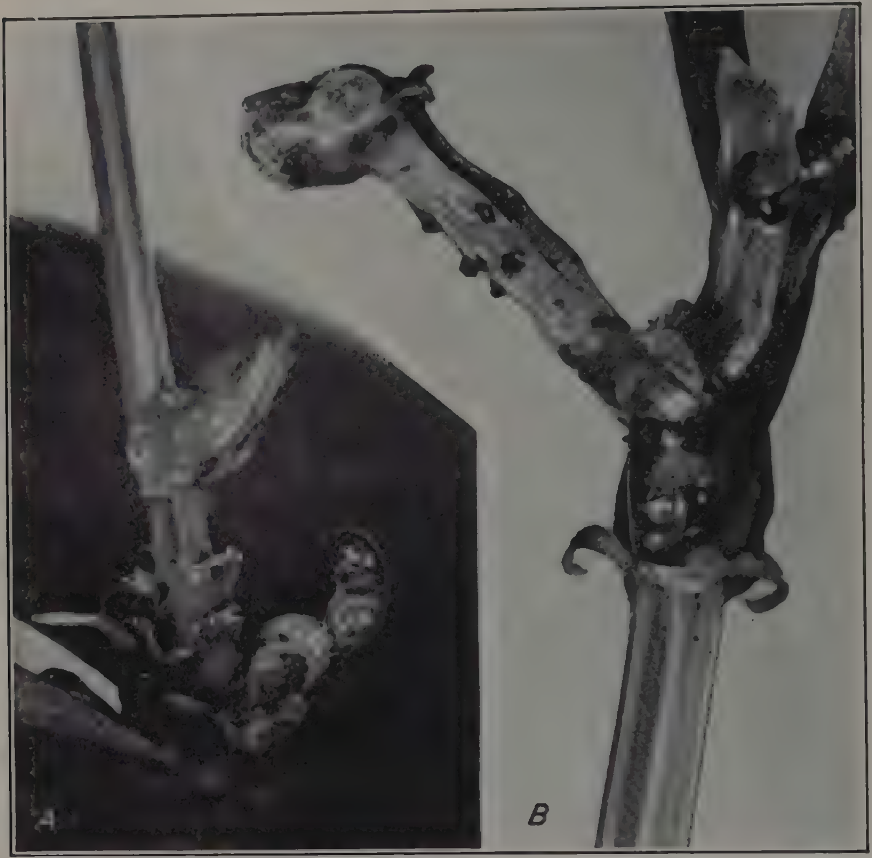

FIG. 6. A. *H. marioni* injury to cowpea infested heavily 36 days earlier, on the very young bud. The apical bud was blinded, the new shoot having grown from a lateral bud. At the right is the first trifoliate leaf reduced to merely petiole and terminal leaflet petiolule by early abscission of leaflets. ×4. B. *H. marioni* injury and egg masses on cowpea 50 days after infestation. Some egg masses, on petiole at left, have broken through the epidermis; others on petiole and stem were beneath the epidermis. Abscissed leaflets also were infected. Observe short, swollen internodes, and much swollen petiolule of abscissed terminal leaflet. Simple leaves were cut off before photographing. ×4.

Infestation without a supporting medium results in the infection of only very young leaves and the stem tip, the larvae apparently being able to penetrate only where embryonic structures are closely appressed. With soil or black sand to support the larvae, however, infection frequently is most abundant in a trifoliate leaf that is 3 to 5 mm. long when infested, with less in the next younger, and almost none in still younger parts. The uppermost, and oldest, parts of these relatively large leaflets frequently are less involved. Heavy infection at this stage results in profuse galling but in relatively mild deformity of leaves (Fig. 5, A and B).

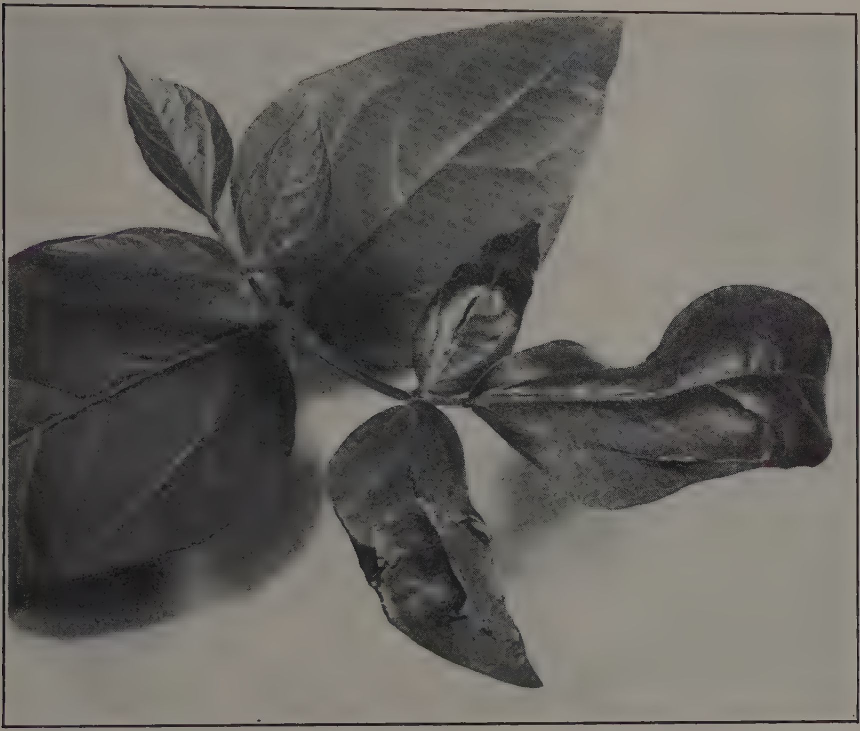

FIG. 7. Distortion accompanying moderately light infection localized in the first trifoliate leaf of a cowpea plant infested 22 days earlier by applying *H. marioni* larvae to the very young bud as soon as the simple leaves had separated. Nematodes were counted in this leaf, processed 39 days after infestation, as follows: Left (upper) leaflet, 14 females, 1 male, 11 immature; terminal leaflet, 9 females, 1 male; and right (lower), 8 females, 3 males, 2 immature. Compare with figure 5, A, where 116 nematodes in one leaflet caused little distortion ×0.9.

Much more severe leaf deformity, out of proportion to numbers of larvae present, results from infection of leaves when only 1 to 2 mm. long. With light infection, deformity including deranged venation may occur at points where nematodes are not established (Fig. 7), and with heavy infection leaflets may be limited to miniature, irregular, structures (Fig. 6, A). Here, some additional pathological action distinct from gall formation must be involved.

INFESTATION OF WOUNDS

When larvae in black sand were applied to longitudinal slits in the adaxial surface of rachises of tomato leaves $\frac{2}{3}$-grown, gall formation along wounds was visible within 10 days and, during 3 months, long, continuous, rugose galls developed. Leaves not wounded but otherwise treated similarly developed no galls.

Cowpea leaves, except when very young, were not entered by larvae placed in black sand on the uninjured surface. Where the epidermis was broken, however, larvae entered, but galls were seen to develop following infestation of only very young cowpea leaves. The rather persistent cambium in large veins of the tomato leaf but not in the cowpea midrib probably explains these contrasting results.

Freshly prepared cuttings of 3 plant species were infested by standing 48 hours in infested quartz sand. The cuttings were then washed to remove nematodes from the surface and were planted in separate pots of sand supplied with nutrient solution. In one cutting of a variety of *Coleus blumei* Benth., processed for examination 50 hours after the beginning of exposure, 30 larvae were found, all below the lowermost node, where they were distributed in the cortex and rays but not in the pith. Four cuttings of this species, after 3 months' growth, were all heavily galled at and below the lowest node, and 2 of them were galled through the first internode above, with nematodes present in all developmental stages. Roots also were heavily galled.

When semi-woody cuttings of a variety of *Begonia* (apparently a spotted-leaf derivative of *B. coccinea* Hook.) were treated in the same way, the check and infested cuttings were entirely similar in external appearance after 3 months of growth, showing some callus but no galls on stem or roots. Microscopic observation, however, demonstrated mature females with eggs, as well as young larvae of a new generation, distributed in pith, cortex, and rays of all infested plants.

Cuttings of a hybrid hibiscus (*H. Rosa-sinensis* L. crossed with native Hawaiian species), which were woody at the time of infestation, exhibited abundant callus but few roots after 3 months. Infested and check plants were entirely similar in external appearance, with no visible stem or root galls but, when the infested plants were examined microscopically, females, eggs, and larvae were found rather abundantly in the parenchymatous tissues developed from the cambium.

BIOLOGY OF THE PARASITE AND DEVELOPMENT OF GIANT CELLS

From microscopic examination of stained paraffin sections of cowpea buds and stems and of clove-oil mounts of leaves of several species, some details of parasitism and pathogenesis have been established for *Heterodera marioni.*

Except where wounds are provided, larvae penetrate chiefly into very young structures, commonly entering before stomata are developed, and entering in groups, presumably as a result of special attractiveness of a wound made by an initial penetrant. Once past the dermatogen or young epidermis, they migrate intercellularly until feeding sites are located. These usually are immature veins or advanced procambial strands, establishment elsewhere being extremely rare. Most frequently, after giant cells are distinguishable, nematode heads are located in the metaxylem region. In leaves they are most abundant in midribs and large lateral veins, but some are found in the finest veinlets (Figs. 8, 9). These nematodes exhibit a striking polar orientation, feeding with the head directed up the stem, petiole, or midrib, or towards the periphery of the lamina, if in a lateral vein. Such orientation is characteristic not only in cowpea (Fig. 8) but also in pea, purslane (Fig. 2, A), tomato, and Emilia, the only plants examined for it. Confused orientation is naturally most frequent in veinlets of irregular direction, but it occurs sparingly even in midribs.

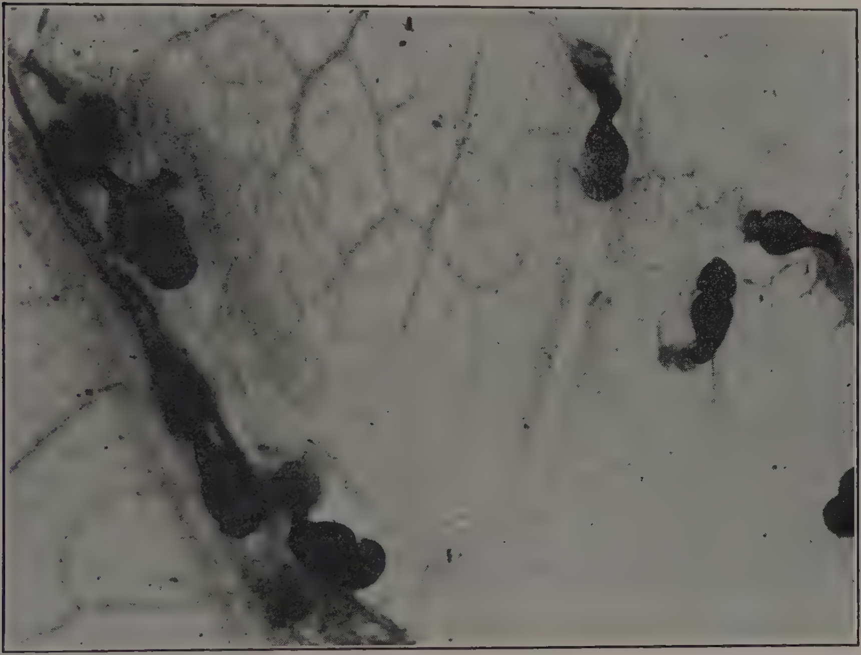

FIG. 8. Piece from lower right of leaflet shown in figure 5, A, illustrating development of females to larger size in large than small veins. The right end of this figure was near the basal margin of the leaflet, at the right of the midrib. Note polar orientation on large vein at left. Compare with figure 9. × 20.

Migration of larvae before locating to feed may continue during several days. In cowpea leaves several millimeters long, larvae have been found feeding after only 2 days. In buds fixed 3 to 5 days after infection occurred, some larvae were feeding in young veins, but those found in undifferentiated tissues of the apical primordium were still migrating. Here they were irregularly oriented in varied postures, contrasting with the relatively straight posture assumed at sites of permanent feeding. That they were still migrating was evidenced by specimens found with either head or tail in the stem tip and with the other end in a very young leaf or stipule primordium. It appears that intercellular migration continues until the larva finds a well formed procambial strand or immature vein, and that, in primordial structures, this migration may be of sufficient extent to cause the deformity of leaves that follows infection of very young buds.

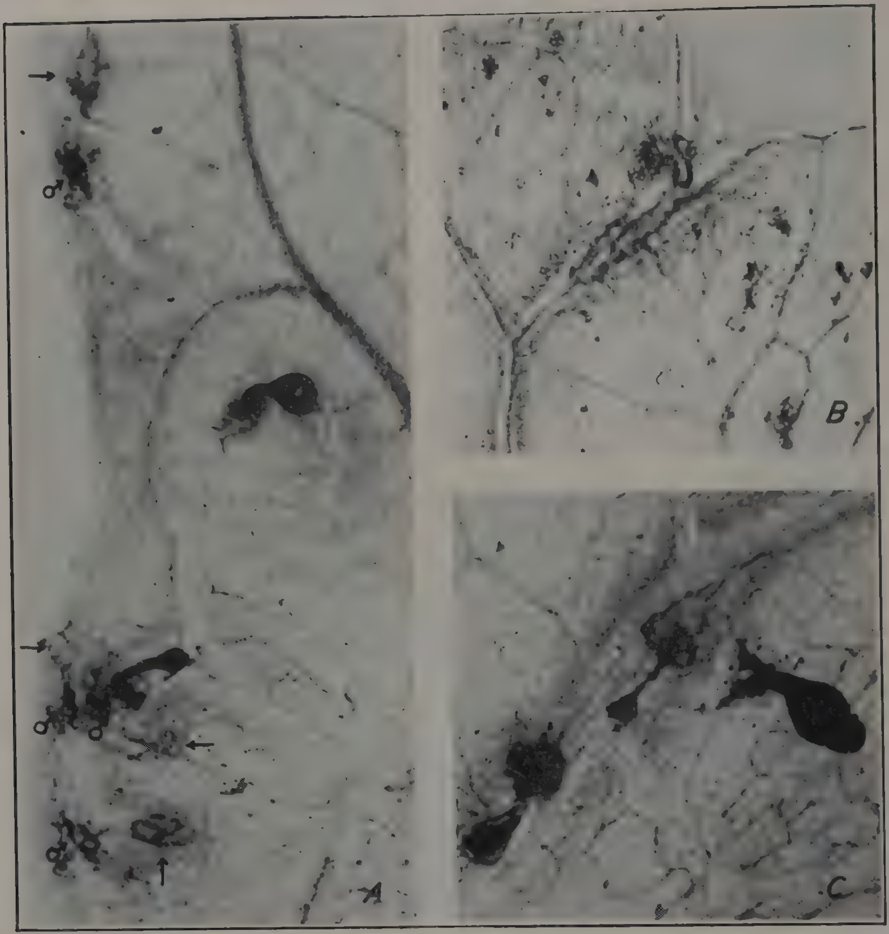

FIG. 9. Nematodes and giant cell groups in pieces of the leaflet shown in figure 5, A. A. Piece from middle left margin, containing 4 males and 2 females, one of the latter poorly developed. The males cannot be distinguished in this figure but locations are indicated. Arrows point to giant cell groups, now apparently nearly empty, where males developed. × 20. B. Adult male, partly obscured by necrotic tissue, at edge of elongate gall on vein containing no other nematode. × 24. C. Two nematodes on large lateral vein with body contents partly collapsed; also, large female with egg mass, with head in group of giant cells that branches with the veinlets. × 20.

Giant-cell groups that develop in leaves and stems are similar to those described in roots (2). They frequently extend up the vascular bundle farther than down, and, when located at the intersection of fine veinlets, they may branch with the vascular tissue (Fig. 9, C). Cells lying beyond the reach of the stylet of the parasite are affected almost as early as are those fed in by the nematode. In cowpea material, fixed after 3 to 5 days, nuclear division figures, some of them apparently aberrant, are frequent in young giant cells. Conjugate division is indicated by finding all nuclei within a given cell in a similar stage of division: as many as 4 metaphase figures were seen in one cell.

Rate of development of females and the size they attain at maturity are related to the feeding site (Fig. 8, 9). In stems, development appears to be almost as rapid as in roots. In midribs and large lateral veins, however, it is somewhat slower, and in veinlets, especially near leaf margins, it is slower still. Here the females mature at smaller size. Where development of females indicates poor nutrition, occasional nematodes have been observed, in association with collapsed giant cells, that apparently died while immature (Fig. 9, C). Males also are more numerous here, agreeing with the findings of Tyler (8) that unfavorable conditions increase the percentage of males. Possibly indicative of poor nutrition in the cowpea leaf in general is the fact that the leaflet shown as figure 5, A, contained 72 females, 26 males and 18 undifferentiated or dead nematodes, representing an exceptionally high proportion of males. Here, males formed definite galls (Fig. 9, B). When males, after their final molt, migrate through the leaf, their irregular path is marked by cell destruction and necrosis.

DISCUSSION

The demonstrated ability of the root-knot nematode to develop to maturity in leaves and stems indicates that some factors other than nutritional adaptation normally limit this root pathogen to subterranean parts. While admitting that occasional leaf and stem galls may have been overlooked or

TABLE 1.—*Representative parasites of aerial plant parts and of roots, compared as to ratio of length divided by greatest diameter*[a]

Nematodes	Stage[b]	Alpha[c]
Parasites of aerial parts:		
Anguina tritici	Larva, 2nd stage	47
Anguina agrostis	Larva, 2nd stage	46
Anguina graminis	Larva, 2nd stage	46
Ditylenchus angustus	Female	58
Ditylenchus dipsaci	(Female)	(41)
Ditylenchus graminophilus	(Female)	(29)
Tylenchus balsamophilus	(Female)	(31)
Anguina millefolii	Larva, 1st stage	21
Aphelenchoides fragariae	Female	52–42[d]
Parasites of roots:		
Ditylenchus radicicola	(Female)	(24)
Heterodera marioni	Larva, 1st stage	32.4
Heterodera schachtii	Larva, 1st stage	20–23[d]
Pratylenchus aberrans	Young female	25
Pratylenchus pratensis	Female	24, 25[d]
	Larva	19
Pratylenchus musicola	Female	30
Rotylenchus similis	Female	28
Rotylenchulus reniformis	Young female	19
Tylenchulus semi-penetrans	Young female	18

[a] Compiled and calculated from divers sources.

[b] Infective stage where data are available; entries in parentheses are not important infective stages.

[c] Length divided by greatest diameter; mean of published values, or calculation from means of published dimensions.

[d] Ranges of 2 or more races.

incorrectly diagnosed, it must be accepted that this nematode, in nature, rarely develops in aerial organs that have not been in contact with soil.

The problem appears to be one of inability of the larvae to gain access to aerial parts. The extreme sensitivity of the *Heterodera marioni* larvae to desiccation and irradiation (4) appears most important, for it limits periods of larval migration over exposed plant surfaces to periods of continuous wetness. In contrast, shoot-infesting nematode species generally develop stages tolerant of desiccation. Special tropisms may help to determine the plant parts selectively attacked by various nematodes. Chlorophyllous tissues, however, are attractive to *H. marioni* larvae (5), and these studies show no lack of stimulus to penetrate such tissues. Another possibility relates to the shape of a nematode in relationship to its ability to traverse moist plant surfaces without such support as soil affords. Presumably, an elongate, slender nematode could more readily overcome surface films to glide from droplet to droplet on a moist leaf or stem than could one proportionately shorter. The compilation of length-diameter ratios of nematodes (Table 1) tends to support this view, for parasites of roots as a group are not nearly so slender as are several of the species most successful as parasites of aerial parts.

Contrasting sharply with the wide adaptability of *Heterodera marioni* to divers hosts is the apparent precision with which it selects its point of feeding in differentiating shoot tissues, almost always in a young vascular bundle and most frequently in the metaxylem region. That this is not merely a selection of immature or meristematic tissues is indicated by the usual failure of these larvae to establish in the apical primordium of the stem or in very young leaf primordia, prior to the beginning of vascular differentiation. Associated with this is the polar orientation of larvae at the time they come to rest, suggesting that the directing stimuli may be related to processes of translocation through immature vascular elements.

Mechanical injury to apical meristems of roots may contribute more to the formation of terminal galls than has usually been assumed, for such injury in the shoot primordium appears to cause both blinding of the bud and severe leaf deformity, even when relatively few larvae are present compared with numbers that may enter somewhat older leaves without causing severe deformity. Although the intercellular migration of these larvae through meristematic tissue appears to kill very few cells, the mere separation of cells probably would suffice to disturb the coordination of tissue differentiation.

That pathogenic effects of secretions of *Heterodera marioni* are not strictly localized around the feeding parasite is demonstrated by these studies. Clearing of veins in laminae of cowpea leaflets may extend several millimeters from the nearest parasite, much farther than the longest giant-cell group. Apparently, it represents mild hypertrophy in or along the vascular bundle similar to the hypertrophy of parenchyma in gall development. Perhaps these cleared veins are to be regarded as greatly elongated galls. The substances stimulating this hypertrophy, however, apparently diffuse some distance along the vascular bundle. Also, the development of typical giant cells out of reach of the mobile head of the nematode proves that secretions of this parasite may diffuse from cell to cell in highly effective concentrations. Such findings call attention to the possibility that very heavy but localized infections, as in roots, may result in at least mild systemic disturbances of plant growth and function, in addition to the more obvious curtailment of root growth and restriction of translocation through galled steles.

The special phase of wound penetration presented by cuttings may assume practical significance in relationship to dissemination of the root-knot nematode, since it is shown that certain cuttings, if exposed to moist, infested media, may develop internal stem infections that cannot be detected at a stage when root growth is just sufficient for the plants to be potted or set out.

Among plants in which no leaf nor stem infection was obtained, *Crotalaria spectabilis*, *Erigeron albidus* and *Euphorbia hirta* have appeared highly resistant to root infection in local trials. *Bixa orellana, Brassaia actinophylla* and *Vernonia cinerea* have developed some root galls in which females matured, but still appear relatively resistant, but *Raimannia odorata* is definitely susceptible to root infection. These data suggest that some plants possess a type of resistance to the root-knot nematode that is not localized in roots alone.

SUMMARY

When larvae of *Heterodera marioni* were placed into buds of favorable plants and protected from drying, penetration occurred and galls developed in which one or more generations of nematodes matured. In some other plants, including species both resistant and susceptible to root knot, no shoot galls were obtained.

In cowpea leaves infected in the bud while very young, galling was accompanied by distortion, crinkling, mild mottling, vein clearing, and occasional enations. Tomato leaves, and the stems of both tomato and cowpea, developed large galls under the influence of successive generations of parasites.

Leaves too mature for the larvae to penetrate unaided were entered readily through fresh wounds. After wound infestation, no nematodes established in half-grown cowpea leaves, but rachises of $\frac{2}{3}$-grown tomato leaves developed large galls. Wound penetration permitted establishment of the parasites in the bases of both soft and woody cuttings.

After penetrating young leaf or stem tissues, larvae migrate to young vascular bundles to feed usually in the metaxylem region, and show strong polarity, nearly all heads being directed up the axis or towards the apex or margin of a lamina. Giant cells develop as in roots.

Males are relatively abundant in cowpea leaves, especially on smaller

veins and near margins where weak development of females indicates poor nutrition.

Among factors normally limiting *H. marioni* to roots, sensitivity to desiccation and irradiation appears most important. Also, data compiled from various sources show these larvae, like infective stages of other root parasites, to be relatively shorter than several successful parasites of shoots; and body shape may influence ability to move over moist surfaces of leaves and stems.

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LITERATURE CITED

1. BUHRER, EDNA M. Additions to the list of plants attacked by the root-knot nematode (*Heterodera marioni*). U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. 22: 216–234. 1938.
2. CHRISTIE, J. R. The development of root-knot nematode galls. Phytopath. 26: 1–22. 1936.
3. GODFREY, G. H. The demonstration of plant-parasitic nematodes in host tissues. Phytopath. 25: 1026–1030. 1935.
4. ————, and HELENE MORITA HOSHINO. Studies on certain environmental relations of the root-knot nematode, *Heterodera radicicola*. Phytopath. 23: 41–62. 1933.
5. LINFORD, M. B. Attractiveness of roots and excised shoot tissues to certain nematodes. Proc. Helminth. Soc. Wash. 6: 11–18. 1939.
6. STEINER, G. The root-knot nematode attacking stems and leaves of plants. (Abstract) Phytopath. 30: 710. 1940.
7. ————, EDNA M. BUHRER, and ARTHUR S. RHOADS. Giant galls caused by the root-knot nematode. Phytopath. 24: 161–163. 1934.
8. TYLER, JOCELYN. Reproduction without males in aseptic root cultures of the root-knot nematode. Hilgardia 7: 373–388. 1933.
9. ————. Proceedings of the root-knot nematode conference. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr., Supp. 109. 1938.

TRANSMISSION OF PLANT VIRUSES BY DODDER

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INTRODUCTION

One of the most effective methods for the transmission of plant viruses is that of grafting. It has been employed in the study of plant viruses that are not readily transmitted by mechanical means or for which no insect vectors are known. The method, however, has obvious limitations, since only plants closely related taxonomically can be grafted successfully. Bennett's report (1) of transmission of sugar-beet curly-top and cucumber-mosaic viruses by dodder (*Cuscuta subinclusa* Dur. and Hilg.) suggests a possible means of overcoming the limitations of the grafting method. All members of the genus *Cuscuta* are twining, scale-leaf plants, mostly devoid of chlorophyll and capable of parasitizing numerous kinds of plants. The parasite loses its connection with the soil and obtains its water, minerals, and elaborated food by the production of haustoria, which penetrate the epidermis and become attached to the conducting tissues of the host. When a single dodder plant parasitizes two adjacent hosts, a natural bridge of conducting tissue, apparently as intimate as a graft union, is established between the two plants (3, 5, 6, 7). In this way, connections in nature may take place between plants as far removed from each other taxonomically as are ferns and asters.

The present paper reports the results of a study on transmission of several different plant viruses by *Cuscuta campestris* Yuncker.[1]

MATERIALS AND METHODS

Virus Stocks

The following viruses were studied: aster-yellows virus (*Chlorogenus callistephi* var. *vulgaris* H.),[2] curly-top virus (*C. eutetticola* H.), tomato-bushy-stunt virus (*Marmor dodecahedron* H.), cucurbit-mosaic virus (*M. astrictum* var. *aucuba* H.), tobacco-mosaic virus (*M. tabaci* var. *vulgare* H.), tobacco-ringspot virus (*Annulus tabaci* var. *virginiensis* H.), and the complex that was previously known as white-clover-mosaic virus (white-clover virus 1) (4), but that is shown in this paper to consist of two viruses.

The Parasite

Cuscuta campestris is native to the United States; it also has been found in South America, Europe, Africa, Asia, and in the Islands of the Pacific Ocean. Its seed probably has been carried with that of clover and alfalfa from one country to another. Not less than 26 genera in 10 plant families are known to have been parasitized successfully by this species, and it is likely that nearly all herbaceous plants may serve as favorable hosts.

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[1] The writer is indebted to Dr. H. L. Dean of the State University of Iowa for supplying the seed, and to Dr. T. G. Yuncker of DePauw University for his assistance in identifying the species.

[2] The Latin names used in this paper follow the system of nomenclature in the Handbook of Phytopathogenic Viruses (2).

Dodder seeds were germinated between layers of moistened filter paper in Petri dishes and the seedlings placed in contact with young, healthy alfalfa and clover plants. Subsequent growth of the parasite on these and similar plants constituted a stock supply.

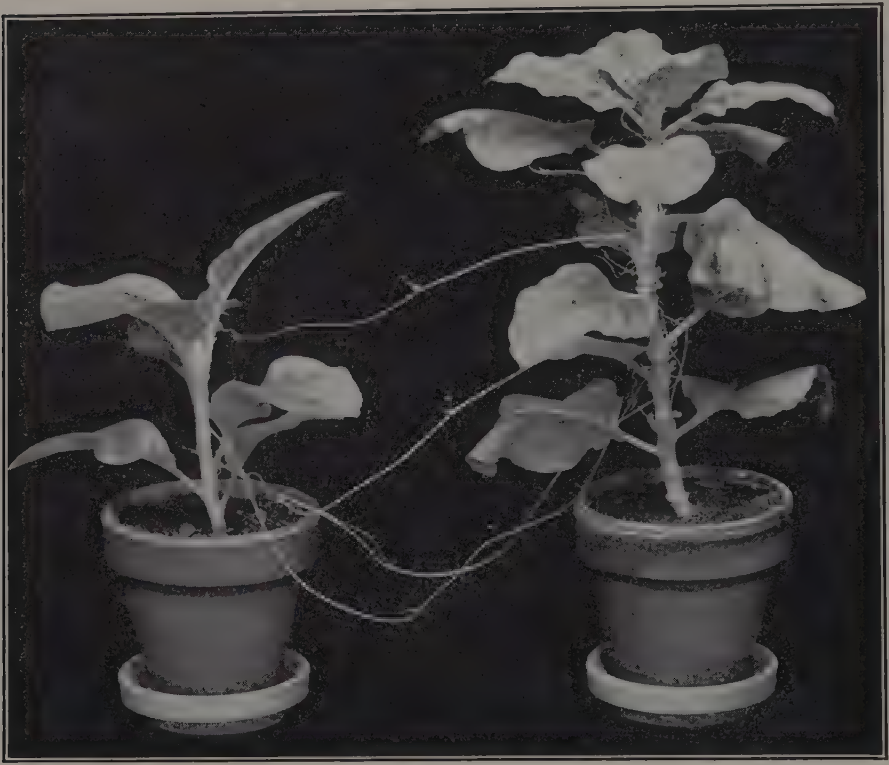

FIG. 1. Method of transmitting virus by the parasitic activities of *Cuscuta campestris* Yuncker. Healthy tobacco plant on left is joined by dodder to an older, diseased plant on the right. Picture taken 3 days after healthy plant became parasitized. (Photograph by J. A. Carlile.)

One- to 2-in. sections were cut from the tips of healthy dodder stems and placed in contact with plants infected with the viruses to be studied. When parasitism was established, 1 to several dodder stems 10 or more inches in length were allowed to become attached to adjacent healthy test plants (Fig. 1). No part of the diseased host was allowed to come in contact with the healthy test plants. The dodder connections were allowed to exist until the test plants became diseased or until it was evident that no symptoms of disease would appear, after which the dodder was trimmed off and the test plants allowed to grow for further observations until the experiment was discontinued. Constant trimming and pruning of the parasite was necessary in certain cases to prevent a growth that would kill or seriously injure its hosts.

A second method of transmission was to parasitize healthy test plants with 2-in. sections from the tips of dodder stems that had grown on diseased hosts. Transmission of virus was judged by the production of disease symptoms or by its recovery from test plants.

All experiments were conducted in a greenhouse, fumigated regularly to destroy insects, and where optimum conditions for rapid growth were maintained. The tests were conducted in the fall and winter of 1939 and the spring and summer of 1940.

EXPERIMENTAL RESULTS

Aster-yellows Virus

In the first test, 10 young China aster plants (*Callistephus chinensis* Nees) infected with yellows were parasitized by dodder, which soon joined them to an equal number of healthy test plants. Observations were made on the test plants 28 to 30 days after haustoria had penetrated the epidermis, but no symptoms of yellows were observed.

A second test was conducted as follows: Ten parasitized yellows plants were joined to 10 healthy plants. Similarly, 10 parasitized healthy plants were connected to 10 diseased plants. Observations were made for 30 days, but no symptoms of yellows developed. Five test plants from each lot were later caged with leaf hoppers (*Macrosteles divisus* Uhler), carrying the virus, and all took yellows. The remaining noncaged plants remained free from the disease.

In the light of these experiments, it seemed desirable to determine whether dodder parasitizing yellows plants picked up the virus. Considerable quantities of young vigorous dodder stems growing upon plants infected with yellows and upon healthy aster plants were cut and placed in separate insect-proof cages. Sixty nonviruliferous leaf hoppers were placed in each cage. After 2 days' feeding, the insects from each cage were transferred to 10 healthy individually caged aster plants and allowed to remain for 8 days. Some insects died, but enough remained to place from 4 to 5 in each cage. The leaf hoppers were transferred at the end of 8 days to a new set of plants and allowed to remain 4 days, when they were removed and destroyed. As might be expected, none of the asters in the first set of plants took yellows; the incubation period of the virus in the insects had not been completed while they fed on these plants. Four aster plants in the second set became diseased with yellows, while all control plants remained healthy. This result demonstrated that virus was present in the dodder grown on hosts infected with yellows.

Since it was shown that *Cuscuta campestris* picked up the virus from yellows plants, further attempts were made to determine whether it would transmit the virus. Indian tobacco (*Nicotiana rustica* L.) is susceptible to yellows, grows well, and is readily attacked by this species of dodder. Ten healthy plants of *N. rustica* and 10 of aster were each parasitized with a 2-inch stem section of dodder cut from host plants infected with yellows. Likewise, an equal number of healthy plants was parasitized with dodder from healthy plants as controls. The 2 groups of plants were far removed from each other, and the plants in each were spaced to prevent contact. The parasite was permitted to grow at random until all plants in each separated group became joined with numerous connections. The plants were respaced once during the progress of the experiment, and at this time most dodder stems connecting adjacent hosts were broken. However, new growth quickly rejoined the plants. Three asters and 3 *N. rustica* plants became diseased with yellows, while all control plants were free from virus symptoms during the course of the experiment.

These results were confirmed by testing an additional 20 *Nicotiana rustica* plants and 10 healthy asters. The asters were placed between rows of *N. rustica* and all plants were joined by dodder taken from diseased plants. Three of the *N. rustica* plants and one aster became diseased with yellows. The plants that became infected were scattered at random among the parasitized host. No aster leaf hoppers were present in the greenhouse, and several hundred healthy aster plants grown in the same house remained healthy.

These experiments show that *Cuscuta campestris* is capable not only of picking up aster-yellows virus but of transmitting it to healthy aster and *Nicotiana rustica* plants.

Bushy-stunt Virus

Twenty tomato plants (*Lycopersicon esculentum* Mill.), infected with bushy stunt, were parasitized by dodder and joined to an equal number of healthy test plants. Five became infected and showed typical symptoms of bushy stunt; the first plant showed symptoms 23 days after haustoria had penetrated the epidermis.

Cucurbit-mosaic Virus

Twenty-two healthy cucumber plants (*Cucumis sativus* L.) were connected by means of dodder to an equal number of plants infected with cucurbit mosaic. The plants grew slowly, but one showed typical cucurbit mosaic 21 days after it became parasitized, and within the next 6 days a total of 7 plants was infected. The remainder of the plants failed to become diseased.

Curly-top Virus

In the first experiment with sugar-beet curly top, 10 young sugar-beet plants (*Beta vulgaris* L.) were tested as described above. The first plant became diseased 19 days after dodder had joined it to an infected host, and a total of 6 plants subsequently became diseased. A second test was conducted during the time when high summer temperatures prevailed in the greenhouse. During this period the parasite grew slowly and branched profusely into small spindly stems. Of 10 infected plants parasitized by dodder, only 6 could be joined to healthy test plants because of the abnormal growth of the parasite. One of the 6 plants became diseased.

Tobacco-mosaic Virus

Of 26 healthy tobacco plants (*Nicotiana tabacum* L.) connected by means of dodder to an equal number of plants affected with tobacco mosaic, 13 became diseased. The shortest time for symptoms of mosaic to appear in any plant was 26 days and the longest time 35 days.

The virus was recovered from dodder that had parasitized mosaic-infected plants. Ten tobacco plants were inoculated with juice extracted from dodder growing on diseased plants, and a similar number of plants was inoculated with juice expressed from dodder parasitizing healthy plants. Nine of the 10 plants in the first group became diseased, while all control plants remained healthy. These results with *Cuscuta campestris* are of special interest in view of Bennett's inability to transmit tobacco-mosaic virus by means of *C. subinclusa* (1).

It seemed of interest to determine whether tobacco-mosaic virus multiplied in tissues of *Cuscuta campestris*. Twenty 2-in. tips of dodder stems were cut from tobacco plants infected with mosaic and allowed to parasitize healthy alfalfa (*Medicago sativa* L.), a plant that is considered immune from the virus. Haustoria penetrated the plants and the parasite soon became established. After 35 days, 20 terminal sections were cut from the dodder on the alfalfa and transferred to a second set of alfalfa plants. Good growth of the parasite on the first set of alfalfa was not produced until 45 days after the plants became parasitized, when a portion of the dodder was removed and ground in a sterile mortar. The juice was used as inoculum for 20 leaves of *Nicotiana glutinosa* L. and 10 tobacco plants. No symptoms of disease resulted from these inoculations. The dodder growing on the second set of alfalfa plants was removed 33 days after the parasite had established itself, ground in a mortar, and tested for virus. The inoculations failed to reveal its presence. These results indicate that tobacco-mosaic virus does not multiply in the tissues of *C. campestris,* although it is transmitted by the parasite.

Tobacco-ring-spot Virus

Twenty-two tobacco plants affected by tobacco ring spot in a chronic stage and 10 plants showing an acute stage of the disease were connected to an equal number of healthy plants by means of dodder. The test plants joined to the hosts in the chronic stage were observed for 33 days and those joined to acutely affected plants were held for a period of 41 days. No symptoms of ring spot developed in either set of plants, and repeated attempts to recover virus from them failed. All were susceptible to infection by juice inoculation. The results showed that tobacco-ring-spot virus was not transmitted by dodder.

The question arose, however, whether virus was present in dodder that had parasitized the ring-spot plants. A considerable quantity of the parasite that had maintained itself throughout its period of growth upon diseased tobacco was ground in a sterile meat chopper. The juice obtained was passed through 2 layers of cheesecloth and used to rub over leaves of 10 healthy tobacco plants. A similar number of plants was inoculated with juice from the hosts upon which the dodder grew. Plants rubbed with juice from *Cuscuta* failed to show symptoms of ring spot, but all plants inoculated with host extract became diseased. This test was confirmed in 3 different experiments. It is concluded that very little or no virus was present in the tissues of the parasite.

White-clover Virus 1[3]

This virus was found by the writer to produce symptoms of streak in pea corresponding to those reported in the literature (4, 8, 9). Several attempts to join healthy pea plants by means of dodder to mosaic-infected white clover failed. The connections in pea did not grow well, and for this reason other hosts susceptible to the white-clover virus were used. Broad bean (*Vicia faba* L.) became systemically infected with the virus and was also a favorable host for *Cuscuta campestris.* Twelve plants of broad bean were joined in the usual way by dodder to white clover (*Trifolium repens* L.) infected with mosaic. Similarly, 8 hop-clover plants (*Medicago lupulina* L.), infected with the virus from white clover, were connected to 8 healthy hop clover plants. One broad-bean plant became mottled 23 days after parasitism was established, and 12 days later a total of 9 plants showed mosaic symptoms. Five hop-clover plants became systematically infected with virus between 30 and 40 days after haustorial penetration.

TABLE 1.—*Results of virus transmission by the parasitic activities of dodder* (*Cuscuta campestris*)

Viruses tested	Hosts	No. plants tested	No. plants infected	Days for symptoms to appear
Aster yellows	Aster (*Callistephus chinensis* Nees)	50	4	40
" "	Indian tobacco (*Nicotiana rustica* L.)	30	6	35
Bushy stunt	Tomato (*Lycopersicon esculentum* Mill.)	20	5	23
Cucurbit mosaic	Cucumber (*Cucumis sativus* L.)	22	7	21
Curly top	Sugar beet (*Beta vulgaris* L.)	16	7	19
Tobacco mosaic	Turkish tobacco (*Nicotiana tabacum* L.)	26	13	26
Tobacco ring-spot	" " " "	32	0	......
White-clover mosaic	Pea (*Pisum sativum* L.)	18	0	......
" " "	Broad bean (*Vicia faba* L.)	12	9	23
" " "	Hop clover (*Medicago lupulina* L.)	8	5	30

Pea plants, mechanically inoculated with juice from the infected broadbeans and hop-clover plants, came down with symptoms of mosaic instead of with those of streak. This result also was obtained several times by rubbing leaves of Dwarf Alderman and Hundredfold peas with juice extracted from dodder stems parasitizing mosaic-infected white-clover plants. On the other hand, inoculations of similar plants with juice from the white-clover plants invariably resulted in streak. It was found that what had previously been considered as white-clover-mosaic virus was actually a mixture of 2 distinct viruses. Dodder isolated one constituent by transmitting it, alone, to the broad-bean and hop-clover plants. The other virus was isolated in cowpea (*Vigna sinensis* Endl.) by mechanically inoculating these plants with juice from the mosaic-infected white clover. When the 2 separate viruses were combined and inoculated into pea, typical streak was produced.

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[3] This virus was kindly supplied by Dr. L. K. Jones of Washington State College, Pullman, Washington.

Table 1 summarizes the data on virus transmission by *Cuscuta campestris.*

DISCUSSION

Six of 7 tested viruses were successfully transmitted from diseased to healthy plants by the natural parasitism of *Cuscuta campestris.* In general, there was a varied and prolonged delay in the appearance of disease symptoms in the test plants. This period was considerably longer than the incubation periods that follow mechanical or insect transmission of these viruses. The delay may be due to the fact that several days are required for haustoria to penetrate through the cortex and unite with the vascular elements of the host. Cultural and climatic conditions probably affect the growth of haustoria through the cortex of the host, and these factors also may play an important part in regulating the passage of materials from parasite to host.

Dodder can be employed in transmission studies with viruses not readily transmitted by mechanical means to a wider range of plants than is possible by grafting. However, as judged by the results of this study, the parasite is not so efficient in transmitting viruses as is grafting. The reasons for this are not known. In the graft the scion and stock are closely related taxonomically, but where dodder parasitizes 2 adjacent hosts there are considerable amounts of foreign plant tissue which the virus must pass through. Nothing is known about the effects of dodder cell sap upon virus activity. Bennett (1) found that cucumber-mosaic virus multiplied indefinitely in the tissues of *Cuscuta subinclusa* when parasitizing plants immune from the virus, and that the parasite transmitted the disease to healthy susceptible plants in 90 per cent of the cases tested. It is known that 2 viruses that are transmitted by dodder do not multiply in the parasite. Bennett (1) found that curly-top virus did not multiply in *C. subinclusa,* and it was shown in this paper that tobacco-mosaic virus did not multiply in *C. campestris.* In both instances a low percentage of transmission was obtained. Thus it appears that when viruses multiply in dodder, a higher percentage of transmission is accomplished by the parasite than when no multiplication takes place.

SUMMARY

Experiments are reported demonstrating that the parasitic seed plant, *Cuscuta campestris,* may act as a vector of viruses by its natural parasitic development among plants.

Successful transmission was obtained with viruses causing the following diseases: aster yellows, bushy stunt, cucurbit mosaic, curly top, tobacco mosaic; and with one virus of the complex previously referred to as white-clover mosaic. Tobacco-ring-spot virus was not transmitted from diseased to healthy tobacco by this method.

Neither tobacco-mosaic virus, which was transmitted, nor tobacco-ring-spot virus, which was not transmitted, multiplies in this species of dodder.

The time required for symptoms to appear in the test plants after they were successfully parasitized by infected dodder was greater than that required for these viruses following mechanical or insect transmission.

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LITERATURE CITED

1. BENNETT, C. W. Acquisition and transmission of viruses by dodder (*Cuscuta subinclusa*). (Abstract) Phytopath. 30: 2. 1940.
2. HOLMES, F. O. Handbook of phytopathogenic viruses. Burgess Pub. Co. (Minneapolis). 1939.
3. PEIRCE, G. J. On the structure of the haustoria of some phanerogamic parasites. Ann. Bot. 7: 291–327. 1893.
4. PIERCE, W. H. The identification of certain viruses affecting leguminous plants. Jour. Agr. Res. [U. S.] 51: 1017–1039. 1935.
5. SCHUMACHER, W. Die Absorptionsorgane von *Cuscuta odorata* und der Stoffübertritt aus den Siebröhren der Wirtspflanze. Jahrb. Wiss. Bot. 80: 74–91. 1934.
6. THODAY (SYKES), MARY G. On the histological relations between Cuscuta and its host. Ann. Bot. 25: 655–682. 1911.
7. THOMPSON, JOHN. V. Studies in irregular nutrition. No. 1. The parasitism of *Cuscuta reflexa.* Trans. Roy. Soc. Edinburgh 54: 343–356. 1925.
8. ZAUMEYER, W. J., and B. L. WADE. Mosaic diseases affecting different legumes in relation to beans and peas. Phytopath. 23: 562–564. 1933.
9. ————, and ————. Pea mosaic and its relation to other legume mosaic viruses. Jour. Agr. Res. [U. S.] 53: 161–185. 1936.

TRANSMISSION OF THE DUTCH ELM DISEASE PATHOGEN BY SCOLYTUS MULTISTRIATUS AND THE DEVELOPMENT OF INFECTION

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It has been demonstrated that elm bark beetles (family Scolytidae) transmit the pathogen, *Ceratostomella ulmi* (Schwarz) Buisman, to healthy elm trees (4, 5). Fransen and Buisman included the lesser European elm bark beetle, *Scolytus multistriatus* Marsham, in their experiments and C. W. Collins *et al.* (2), working in the United States, also obtained transmission to healthy trees by this species. Inoculations made during the early part of the growth period resulted in a higher percentage of infections than those made in middle or late summer (5). Fransen (4) considered that he was able to trace infection to certain feeding scars made by scolytid beetles. May (7) found that *C. ulmi* could be isolated from scars from which short brownish streaks extended into the twig and from scars "in which no apparent infection had occurred."

Coremia of *Ceratostomella ulmi* have been observed in maternal tunnels and pupal cells of both *Scolytus scolytus* F. and *Scolytus multistriatus* (6, 8). Fruiting structures (mostly coremia) have frequently been observed in the maternal galleries and pupal cells of *S. multistriatus* in diseased trees in and around New York City. Also, they have been observed in dead wood from trees not killed by the Dutch elm disease, and in healthy logs exposed to beetle attack in the field. A discussion of this and a review of the literature are given by Donald L. Collins *et al.* (3). The beetles become infested with the fungus, mostly on their outer surfaces, from the coremia in the pupal cells. Also, the fungus has been isolated from their intestinal tracts (1).

MATERIALS AND METHODS

Cultures of Beetles

1. To make isolations of the pathogen from the surfaces and from the intestinal tracts of beetles they were collected singly in sterile test tubes. The individual adult beetle was then: (a) immersed in a sterile drop of water in a Petri dish for 30 minutes, (b) immersed in a 1 to 1,000 mercuric bichloride solution for 2 minutes, and (c) rinsed in sterile water. The intestinal tract was then removed and macerated in a drop of sterile water in a Petri dish. Acidified potato-dextrose agar was poured into the plates

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[1] Thanks are due the Boyce Thompson Institute for Plant Research for making available facilities for doing much of the work reported here. Dr. W. H. Rankin, in charge of Dutch Elm Disease Control for the New York State Department of Agriculture and Markets, and members of his staff, assisted in locating and collecting material. Dr. D. S. Welch made suggestions regarding the work and critically read the manuscript. Others who assisted in the work are L. L. Pechuman, Seth Pope, and Henry Dietrich.

containing the surface washings and the intestinal tracts. It may be pointed out that the method of dissection obviated any chance of contamination of the intestinal tracts from the few spores that might have escaped the mercuric bichloride treatment.

2. To determine the fungus load carried by beetles used in certain of the transmission experiments the beetle-infested logs were first placed in small screen cages. Soon after emergence had begun beetles were collected daily under aseptic conditions, each in a separate sterile test tube. Each beetle was macerated in a drop of sterile water in a Petri dish, one dilution made, and acidified potato-dextrose agar poured into the plates.

Cultures of Plant Tissues

The method as described was used both for field-collected and experimental material. The experimental trees were completely dissected in searching for infected tissue. If any brown discoloration was found in the wood some of the discolored tissue was excised and planted on potato-dextrose agar in Petri dishes. If discolored tissue was not found each feeding scar made by the beetles was cultured separately. If only a small amount of discolored tissue was present, both it and the scar were cultured, but in separate plates. Where the discolored tissue involved more than one scar, the scars were not cultured. The recovery of *Ceratostomella ulmi* from discolored (brown) wood was considered as proof of infection. Where the fungus was recovered from the scars only, it was considered that infection had not taken place, but that transmission of the fungus by the beetles had occurred. On this basis a distinction between "infection" and "transmission only" is made in the tables.

Methods of Inoculation

1. *By Naturally Infested Beetles.* Wood, producing beetles infested with *Ceratostomella ulmi,* was enclosed with healthy trees in out-of-door cages 2½ feet wide by 5 feet long by 6 feet tall. The side walls were of wire screen and the roof was of glass. Eight trees were placed in each cage. After allowing periods of 3 to 10 days for the beetles to emerge, the logs were removed.

2. *By Artificially Infested Beetles, in the Study of Development of Infection.* The beetles were collected and immediately surface-disinfested as follows: They were (a) dipped in water, (b) in 50 per cent ethyl alcohol, (c) immersed in 1 to 1,000 mercuric bichloride solution for 2 minutes, and (d) rinsed 3 times in sterile distilled water. After this treatment the beetles were placed on filter paper in sterile Petri dishes to dry. After drying they were placed in large sterile test tubes with elm twig cultures of *Ceratostomella ulmi* bearing numerous coremia and conidiophores. After being allowed to crawl over the cultures the beetles were taken out and placed on healthy trees. To do this a cage made of celluloid closed at each end with muslin was fastened over the top of the tree and 5 beetles were

placed in the cage. The trees in experiments 1 and 3 were inoculated out-of-doors and those in experiment 2 were inoculated in the greenhouse. The latter were moved out-of-doors in November following their inoculation in August. Otherwise, all trees were held in screen or cheesecloth cages out-of-doors during the summer months until they were examined. During the winter they were all stored out-of-doors.

Experimental Trees

With one exception the trees used in all experiments were 2-year-old budded nursery stock, 6 to 8 feet tall. They were potted in the fall for use the following spring. In experiment 1 of the study of development of infection, 40 of the trees inoculated were 3 years old and had been potted in the spring of the year previous to their use. All trees were in fair to good vigor at the time they were inoculated and, in general, continued extension of shoot length until about July 1.

RESULTS OF CULTURES

Isolations from Beetle Intestinal Tracts

In order to determine whether *Ceratostomella ulmi* occurred in the intestinal tracts of *Scolytus multistriatus*, cultures were made from beetles as they emerged from wood taken from diseased trees. Of 669 such cultures, 509, or 76.1 per cent, produced the fungus from surface washings and 96, or 14.3 per cent, from the intestinal tracts. The number of colonies in plates made from the intestinal tracts was much smaller than in those from surface washings.

Isolations from Beetle-feeding Scars Collected in the Field

In September, 1935, branches bearing feeding scars of scolytid beetles were collected from apparently healthy elm trees in an area of severe disease incidence. These branches were brought to the laboratory and cultures made of the scars. The fungus was isolated from a short discolored streak near one scar. In addition to this it was obtained from 10 of 1,102 scars cultured where no invasion was found beyond the scar tissue. The 10 scars yielding *Ceratostomella ulmi* came from 6 of the 14 trees represented.

On March 24, 1936, similar collections were made from the same location. Of 612 scars cultured *Ceratostomella ulmi* was obtained from 15.

In 1937 and in succeeding years cultures were made of similar material collected at several other locations. *Ceratostomella ulmi* was obtained from scars from most collection stations located near points where trees infected with the Dutch elm disease had been found.

RESULTS OF INOCULATION EXPERIMENTS

Transmission by Naturally Infested Beetles

In these experiments, logs used as beetle sources had previously yielded beetles 50 to 65 per cent of which carried spores of the fungus.

One tree in experiment 1 was discarded because of extensive discoloration in the wood not caused by *Ceratostomella ulmi.*

TABLE 1.—*Transmission by naturally infested beetles*

Experiment	Dates trees inoculated	Dates trees examined	Trees			Feeding scars		
			Number examined	Number with transmission only	Number infected	Number cultured	With *C. ulmi*	
							Number	Per cent
1	8 on July 1 and 8 on July 4, 1937	Sept. 17–18, 1937	15	5	5	414	25	6.0
2	8 on May 28 and 16 on June 8, 1938	Aug. 29–31, 1938	24	9	5	291	23	7.9
	Uninoculated	Aug. 31, 1938	10	0	0	....	......	......

At the time the trees in experiment 1 were examined, the fungus had invaded the wood throughout one tree. Most of the leaves had dropped and there was considerable dead wood in this tree. On 3 others 10 to 25 per cent of the leaves were wilted or had dropped. There were several dead twigs and branches. The discolored tissue varied in extent from 10 to 25 inches along the various branches. In some instances the infected twigs had died, the fungus being obtained from the dead tissue.

At the time the trees in experiment 2 were examined, 4 of the infected trees showed leaf symptoms such as defoliation, drying, and green wilt. On 1 of these trees most of the branches were dead and there were several dead twigs on the other 3 trees. In 2 trees most of the wood was discolored; in others there was less discoloration. In 1 tree the only discolored tissue was in 1 new shoot, with about 12 inches of the tip involved.

Development of Infection

In 1936 and 1937 experiments were designed for the purpose of determining whether, if the trees be left standing, infections may eventually take place in trees bearing scars in which the fungus persists for 1 or more seasons.

Experiment 1. Twenty trees were inoculated on each of the dates June 30, July 1, 2, and 3, 1936, with beetles that had been on the cultures for 2 hours. After the beetles had been on the trees 7 days, they were removed and cultured singly.

Recovery of the fungus from the beetles after their removal from the trees was very good, a few hundred colonies being obtained from each beetle.

Experiment 2. Sixty trees were inoculated, 20 on each of the dates August 26, 27, and 28, 1936. The method was similar to that used in experiment 1.

TABLE 2.—*Development of infection following transmission by beetles*

Experiment	Dates trees inoculated	Dates trees examined	Trees			Feeding scars			
			Number examined	Number with transmission only	Number infected	Number present	Number cultured	With *C. ulmi*	
								Number	Per cent
1	*June 30, July 1, 2,* and *3, 1936*	*July 14–17, 1936*	19	14	0	115	114	31	27.2
		Oct. 13 and *14, 1936*	20	10	6[a]	112	106	37	34.9
		Sept. 6, 1937	19	12	4	124	110	49	44.5
		June 23, 1938	18	13	1	109	94	41	43.6
	Uninoculated	*June 23, 1938*	10	0	0	0	....	......	....
2	*Aug. 26, 27,* and *28, 1936*	*Oct. 2–4, 1936*	20	13	0	172	167	28	16.8
		Sept. 6, 1937	20	16	0	178	157	49	31.2
		June 27 and *28, 1938*	20	15	0	166	149	30	20.1
	Uninoculated	*June 28, 1938*	10	0	0	0	....	......	....
3	*June 15* and *16, 1937*	*July 20, 1937*	18	12	5	73	71	34	47.9
		Sept. 14, 1937	18	9	7	66	63	35	55.6
		July 7, 1938	18	12	5	69	65	36	55.4
		Oct. 19, 1939	28	16	4[b]	104	78	42	53.8
		July 23–25, 1940	28	16	3[c]	104	84	37	44.0
	Uninoculated	*July 25, 1940*	20	0	0	0	....	......	....

[a] Includes a tree that wilted and was examined July 31, 1936.

[b] Does not include 1 tree with a dead branch, which probably had been infected but from which *Ceratostomella ulmi* was not obtained when cultures were made in October, 1939.

[c] Does not include 1 tree that wilted and apparently was invaded throughout by the fungus in 1937 but from which *Ceratostomella ulmi* was not obtained in cultures made from living discolored wood in July, 1940.

Recovery of the fungus from the beetles after removal from the trees was slightly less successful than in experiment 1.

Experiment 3. One hundred twenty trees were inoculated, 60 on June 15 and 60 on June 16, 1937. The beetles were left on the cultures overnight (about 22 hours) and were removed from the trees after 6 days.

Recovery of the fungus from the beetles after removal from the trees was almost as good as in experiment 1.

At various dates, as indicated in table 2, trees from the different experiments were cut and examined. Each lot examined was selected so as to represent the whole as to number of feeding scars.

In experiment 1 only 1 tree wilted. In experiment 2 no wilt occurred. In experiment 3, 1 tree was wilted by July 6, 1937. By August 7, after 18 trees had been cut for examination, wilt had occurred on 5 other trees, varying in extent from $\frac{1}{5}$ to $\frac{3}{4}$ of the leaves on each tree.

DISCUSSION

In the experiments here reported *Ceratostomella ulmi* became established in many beetle feeding scars without the occurrence of true infection. It is shown in table 2 that infection did not occur in the trees inoculated in August, 1936, although the fungus could be isolated from feeding scars on these trees nearly 2 years after inoculation. Cultures made a few weeks, or at various intervals up to 2 or 3 years after the trees had been inoculated, indicated little or no difference in the percentage of scars containing the fungus in viable condition. On the other hand, there appeared to be no initiation of infection after a few weeks following the inoculations. That is, if *C. ulmi* was introduced into a feeding scar by the beetles and invasion of the adjacent tissue did not take place within a few weeks, infection never resulted from that inoculation. The fungus remained viable in the scar tissue on some trees more than 3 years without invading the trees.

In several infected trees in these experiments all discolored tissue was dead at the time of examination. If the cultures were made from such trees within the growing season of the year in which they had been inoculated *Ceratostomella ulmi* was isolated from the dead part but not from the live parts below. In a few instances it was not obtained from the proximal part of the dead portion, although it was viable in the distal part.

There was some indication that the number of trees showing infection by cultural examination 2 or 3 years after inoculation was smaller than when the examination was made earlier. In no case did the fungus invade tissue produced in years subsequent to the year of inoculation.

The results of these experiments and the ease with which the Dutch elm disease pathogen can be isolated from feeding scars collected from trees in the field indicate that a great many inoculations made by the feeding activities of the beetles do not result in true infection.

SUMMARY

Cerastomella ulmi was obtained from the outer surfaces of *Scolytus* *multistriatus* adults in higher percentage than from their intestinal tracts. Likewise, the numbers of colonies in the cultures were much greater.

Transmission of the fungus to potted healthy elm trees by the beetles in their feeding activities was readily obtained. The fungus was reisolated from feeding wounds on uninfected trees as long as 3 years following inoculation.

Infection was obtained on trees inoculated during the late spring and early summer. Inoculations made later in the season induced infection less frequently or not at all.

There was evidence that the fungus sometimes died in infected trees. Sometimes the infected twig died without the entire tree becoming invaded.

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LITERATURE CITED

1. BETREM, J. G. De iepenziekte en de iepenspintkevers. Tijdsch. over plantenziekten 35: 273–288. 1929.
2. COLLINS, C. W., W. D. BUCHANAN, R. R. WHITTEN, and C. H. HOFFMAN. Bark beetles and other possible insect vectors of the Dutch elm disease *Ceratostomella ulmi* (Schwarz) Buisman. Jour. Econ. Ent. **29**: 169–176. 1936.
3. COLLINS, DONALD L., K. G. PARKER, and HENRY DIETRICH. Uninfected elm wood as a source of the bark beetle (*Scolytus multistriatus* Marsham) carrying the Dutch elm disease pathogen. N. Y. (Cornell) Agr. Exp. Stat. Bull. 740. 1940.
4. FRANSEN, J. J. Enkele gegevens omtrent de verspreiding van de door *Graphium ulmi* Schwarz veroorzaakte iepenziekte door de iepenspintkevers, Eccoptogaster (Scolytus) *scolytus* F. en *Eccoptogaster* (Scolytus) *multistriatus* Marsh. in verband met de bestrijding dezer ziekte. Tijdsch. over plantenziekten **37**: 49–62. 1931.
5. FRANSEN, J. J., and CHRISTINE BUISMAN. Infectieproeven op verschillende iepensoorten met behulp van iepenspintkevers. Tijdsch. over plantenziekten **41**: 221–239. 1935.
6. GROSSMAN, HÉLÈNE. Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. Zeitschr. für Parasitenk. **3**: 56–102. 1930.
7. MAY, CURTIS. Outbreaks of the Dutch elm disease in the United States. U. S. Dept. Agr. Circ. 322: 1–19. 1934.
8. WOLLENWEBER, H. W., and C. STAPP. Untersuchungen über die als Ulmensterben bekannte Baumkrankheit. Arb. Biol. Reichsanst. für Land- und Forstwirtsch. **16**: 283–324. 1928.

STEM BLIGHT OF COTTON CAUSED BY ALTERNARIA MACROSPORA

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A stem blight, apparently hitherto unreported, is widespread in the cotton district of Szechuen Province, China. In certain years it may cause substantial loss, especially on certain varieties of Asiatic cotton, *Gossypium arboreum* L. The local farmers are aware of its seriousness, and the name "dry scar," descriptive of its symptoms, is given to it. The practice of the farmers is to eradicate infected plants as soon as they are discovered.

A species of *Alternaria* is found in constant association with this disease. Although species of this fungus are known commonly as causes of leaf spot, boll rot, and seedling blight of cotton, no record can be found, so far as the writers are aware, of its attack on the stem of the mature plant. *Macrosporium gossypium* Thüm., which, according to Mason,[1] is an *Alternaria* allied to *A. tenuis* Nees, was described originally as occurring on dead stems of cotton; but it is rather doubtful whether the fungus is a pathogen. Later, Hopkins[2] provisionally referred a fungus from Southern Rhodesia to this species and, without examining an authentic specimen of the original, made the combination, *A. gossypina* (Thüm.) Hopkins. Yet he made no inoculations on the stem of cotton. A study on the morphology and parasitism of this particular isolate of *Alternaria* causing stem blight of cotton was, therefore, made.

SYMPTOMS OF THE DISEASE

The disease occurs on stem, twig, and leaf petiole of mature cotton plants. At first the infection appears as a dark brown and somewhat circular spot. Under favorable conditions, the spot enlarges rapidly, with its center becoming deeply sunken to form a canker (Fig. 1, A). With age the spot changes gradually to dark gray and becomes elliptical or oval, with its long axis parallel to the stem. The infected tissue usually splits longitudinally or cracks into small pieces (Fig. 1, B). Finally the diseased stem or twig breaks off at the canker, resulting in the death of part or all of the plant (Fig. 1, C). In the field, a cotton plant inoculated artificially at blossoming stage may die in the course of one week.

When the upper part of the petiole is infected, the lesion may extend upward into the midrib and veins near the base of the leaf. The diseased veins turn brown, being similar to the infection by *Bacterium malvacearum* E. F. S. However, the transparency and water-soaked appearance commonly associated with *B. malvacearum* do not occur in the stem blight.

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[1] Mason, E. W. Annotated account of fungi received at the Imperial Bureau of Mycology. List 2, Fasc. 1. The Imperial Bureau of Mycology, Kew. 43 pp. 1928.

[2] Hopkins, J. C. F. *Alternaria gossypina* (Thüm.) comb. nov. causing a leaf spot and boll rot of cotton. Trans. Brit. Myc. Soc. 16: 136–144. 1931.

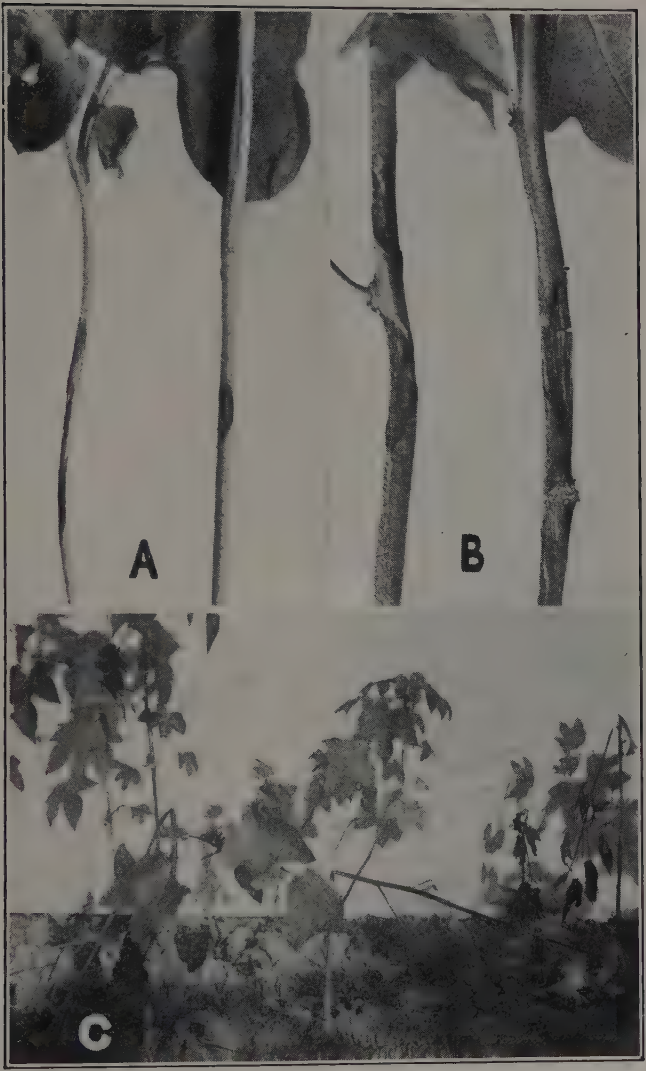

FIG. 1. Stem blight of cotton. A. Stem lesions on seedlings of the variety Trice. B. Advanced stage of stem invasion on a local Chinese variety. C. Portion of cotton field showing the damage caused by the disease.

THE CAUSAL FUNGUS

Pathogenicity

The fungus is readily obtained in pure culture by ordinary isolation technique. Owing to the scantiness of sporulation in culture, inoculation could be accomplished only by applying a bit of mycelium on agar to the stem or upper surface of the leaf. The plant thus treated was kept in a moist chamber, or the inoculated part was bound around with damp cotton-wool. A control plant was similarly treated with only a piece of agar. In all cases the agar was taken off after 1 day. The boll was inoculated by needle pricks. In one series, the inoculated boll was allowed to grow on the plant and was enclosed in a paper bag to keep a higher humidity. In another, it was picked off from the plant and kept in a moist chamber in the laboratory.

The stem of the cotton plant was susceptible to artificial inoculation in all stages of growth. In seedlings, the lesions on the stem develop in 2 to 3 days. On an average approximately 70 per cent of uninjured seedlings became infected, while wounding often increased the infection to 100 per cent. On the mature plant, the incubation period is 1 or 2 days longer than for the seedlings. Seedlings of the American cotton, *Gossypium hirsutum* L., are as susceptible as the local varieties of *G. arboreum,* but, with age, *G. hirsutum* becomes more resistant. For instance, 7 out of 10 plants of the local variety, inoculated during blossoming, were infected in contrast to 4 out of 15 of the American variety Trice.

On the cotyledon and the mature leaf of cotton, the symptoms produced after inoculation appear to be identical with those produced by *Alternaria macrospora* isolated from a leaf spot of cotton. The initial lesion is a minute, circular, and dark green spot. As the spot enlarges the color changes to gray, with or without a dark brown margin.

On cotton bolls of various ages, infection is at first a minute, grayish-brown spot. In the more advanced stage, lesions remain somewhat circular in outline until neighboring ones coalesce, but the color often changes to a purplish tinge. Under extremely humid conditions conidia develop in sufficient quantity to give a black cast to the center of the spot.

Morphology

The fungus sporulates scantily on natural substratum. On potato-dextrose agar, the culture is covered densely with white aerial mycelium, occasionally with a few conidia formed underneath. On the autoclaved cotton stem, sporulation is considerably increased, yet is not abundant. On material of such source the morphological studies of this fungus are based. The conidiophores are brown, septate, usually non-branched, and measure 21–124 × 4–10 μ. The conidia are brown, obclavate, with 3 to 13 transverse and usually 3 to 5 longitudinal septa, constricted at each septation, and provided with long, hyaline, filiform, and septate beaks (Fig. 2). The detailed measurements of conidia of the stem-blight fungus with 3 isolates of *Alternaria macrospora* from leaf, cotyledon, and boll lesions are given in table 1.

Taxonomy

During the course of the present study, a number of isolates of *Alternaria* were obtained from different sources. The detailed account of their comparison in morphology and symptoms produced will be discussed in a separate paper. In brief, all the isolates may be reasonably assigned to two groups, *i.e., A. tenuis* and *A. macrospora.* The former consists of those with shortly beaked condia, usually forming chains of various lengths.

Based upon the morphology of conidia and cultural characteristics, this may be divided further into subgroups. In *A. macrospora,* the conidia are usually provided with long, filiform beaks, rarely form chains, and, as Mason[3] suggested, attain a length of approximately 200 μ. Moreover, their sporulation is usually scanty in agar culture. As no authentic material of *A. macrospora* seems to be in existence, the determination is based merely upon the current conception of this species as held by Mason,[3] Jones,[4] and others. All the isolates from stem blight and boll rot of local cotton are similar in morphology and belong to the *A. macrospora* group. Isolations from leaves, however, yielded both organisms.

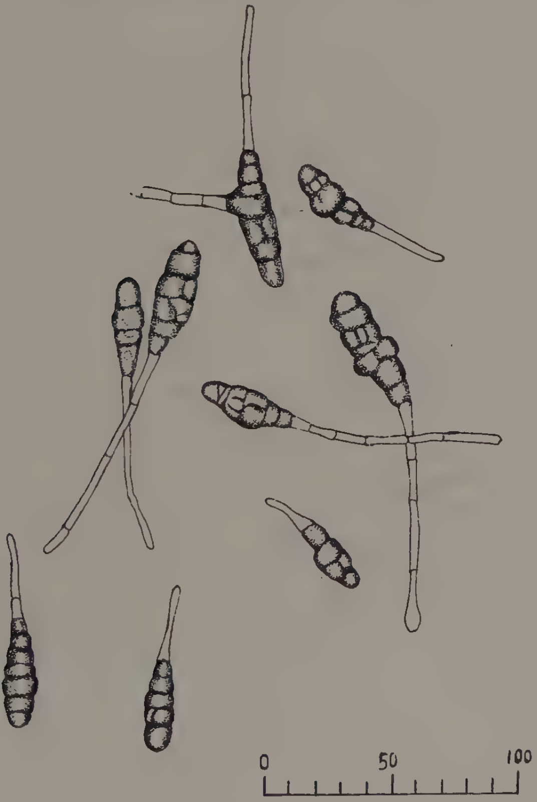

FIG. 2. Conidia of an isolate of *Alternaria macrospora* from stem blight of cotton.

As summarized in table 1, the measurements of the fungus causing stem blight resemble closely those of isolates identified as *Alternaria macrospora* from lesions on leaf, cotyledon, and boll of cotton. Cross inoculation experiments also show that isolates from all sources do not differ in pathogenicity, all being able to attack stem, leaf, cotyledon, and boll. Therefore, they are considered to belong to the same species, *i.e., A. macrospora.* The lack of

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[3] See footnote 1.

[4] Jones, G. H. An *Alternaria* disease of the cotton plant. Ann. Bot. 42: 935–947. 1928.

TABLE 1.—*Measurements of conidia of four isolates of Alternaria macrospora*[a]

Source of isolate	Variety of cotton	Length, in μ						Width, in μ		Number of septa	
		Conidial body		Beak		Total		Range	Mean	Trans-verse	Longi-tudinal
		Range	Mean	Range	Mean	Range	Mean				
Stem	Local	25–113	57.7	15–187	74.7	40–278	121.3	14–28	18.7	3–13	1–9
Leaf	Local	25– 66	43.0	36–124	59.7	69–168	101.0	8–25	13.0	2– 8	0–5
Cotyledon	Delfos 531	29– 68	44.8	32–180	99.7	72–241	144.7	11–22	18.1	3– 8	0–6
Boll	Local	41– 86	61.2	58–216	103.6	101–288	164.7	14–29	20.4	4–10	1–9

[a] All the measurements are based on 200 conidia.

record on this species as a stem pathogen in other countries has two possible explanations: either the local environmental conditions in China are especially favorable for its pathogenic expression on the cotton stem, or there occurs a local strain of the fungus pathogenically different from other allied strains that are commonly regarded as the leaf fungus. In order to clarify this question, it seems desirable to test the parasitism of this species on cotton stems in other parts of the world.

Physiology

The relation of temperature and H-ion concentration of the medium to the mycelial growth of the fungus was investigated. Quadruplicate cultures were grown on potato-dextrose agar in Petri dishes. For temperature relations, cultures were incubated in electrically regulated chambers at intervals of 4°, from 16 to 40° C. The H-ion concentrations of medium were adjusted by the addition of NaOH and HCl after sterilization and were determined colorimetrically. Measurements of diameter of colonies were regarded as the criterion of growth rate.

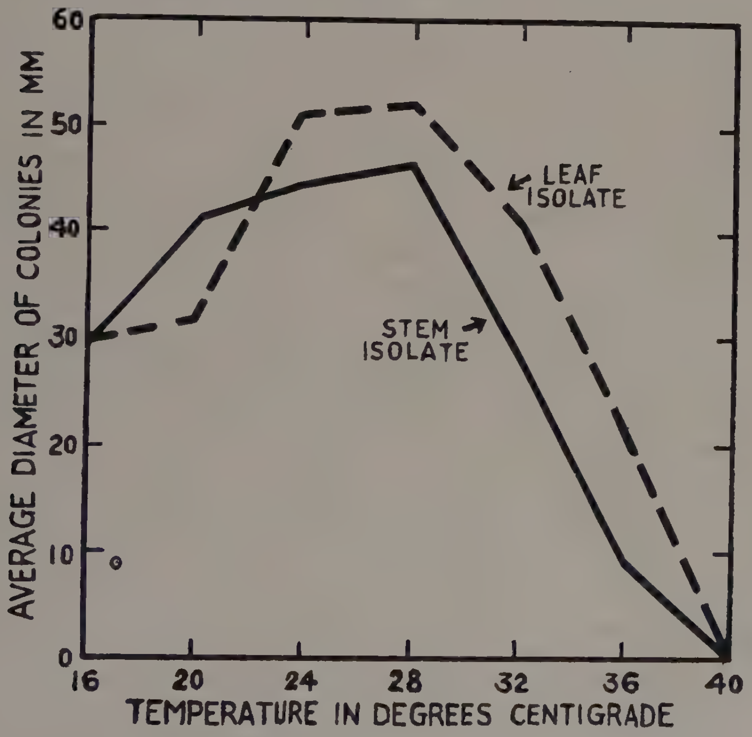

FIG. 3. Relation of temperature to the growth of two isolates of *Alternaria macrospora* on potato-dextrose agar after 10 days.

As shown in figure 3, the two isolates, one from a stem lesion and the other from a leaf spot of the local variety of cotton, behaved similarly regarding reactions to temperature. Growth occurred at temperatures between 16° and 36°, with the optimum at 28° C. The maximum lies somewhere between 36 and 40° C.

For the study of relationship of H-ion concentration, only the isolate from cotton stem was used. The fungus apparently grows over a wide range of pH. The shape of the curve is bimodal, with one mode at the acid side and another at the alkaline side (Fig. 4).

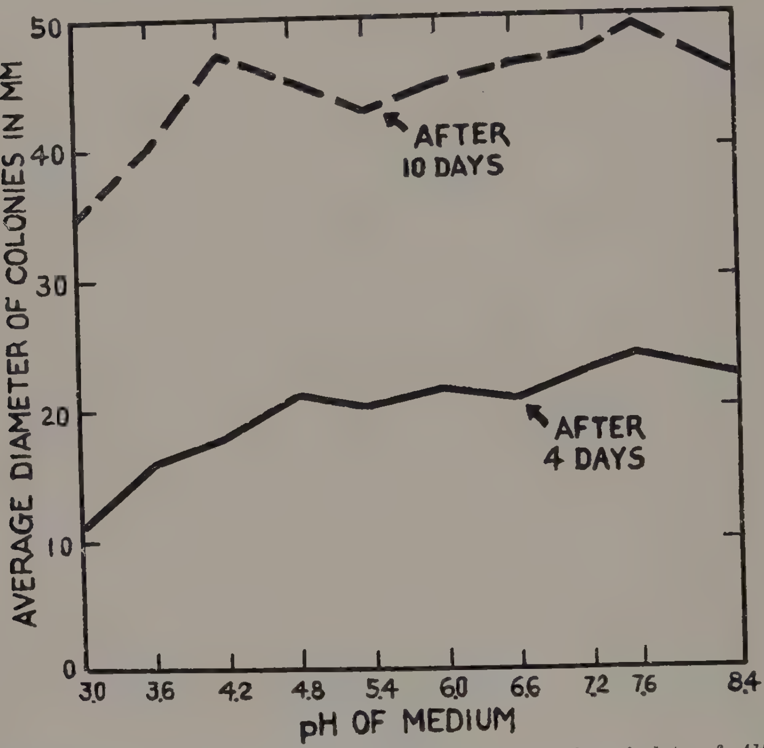

FIG. 4. Relation of H-ion concentration to the growth of an isolate of *Alternaria macrospora* from stem blight of cotton on potato-dextrose agar at 24° C.

SEASONAL DEVELOPMENT OF THE DISEASE

The stem blight occurs chiefly in July just prior to anthesis in cotton. Successive periods of rainfall, high humidity, in combination with fairly high temperature, invariably favor serious outbreaks of the disease. In 1938, a year with excessive rainfall in July, 30 per cent infection was observed in one field; while, in 1939, the disease was greatly checked by the unusually dry weather, and the damage caused was almost negligible. All plants not too severely infected recuperated sufficiently to produce a fair crop in 1939.

The effect of temperature on the infection was experimentally determined by incubating inoculated cotton seedlings in chambers of constant temperatures for 2 days. Since the humidity in the chambers could not be satisfactorily controlled, the results obtained were not considered conclusive. The optimum temperature could, however, be determined as approximately 28° C., the same as the optimum for mycelial growth.

OVERWINTERING

This species of *Alternaria* apparently overwinters chiefly on the infected cotton plants left in the field. Isolations made from old stalks known to be infected during the previous growing season, however, gave unsatisfactory results because of heavy contamination with saprophytic and fast-growing fungi. In October, 1938, cut pieces of infected cotton stem, wrapped in thick paper and wire gauze, were carried through the winter under the following conditions: (1) Hung on the tree in the open; (2) placed on the surface of the ground; (3) buried 5 inches deep in the soil. Isolations were made in May, 1939. The fungus was recovered in every instance from the materials on the tree and on the surface of the ground, but not from that buried underground.

The frequent occurrence of this species of *Alternaria* from surface-sterilized cotton seeds cultured on agar plates also indicates the possibility of seed transmission of the disease.

SUMMARY

A stem blight of cotton is widespread in the cotton district of Szechuen Province, China. The lesion appears as a canker on stem, twig, and leaf petiole of the mature cotton plant.

The pathogenicity of the causal fungus was fully established by inoculation experiments on stem, leaf, and boll of cotton. The fungus has brown, obclavate, muriform conidia, provided with long, hyaline, filiform beaks and borne singly on conidiophores. It was considered as a strain of *Alternaria macrospora* Zimm., morphologically and pathogenically resembling other local strains of the same fungus causing leaf spot and boll rot of cotton.

The fungus grew at temperatures between 16 and 36°, with optimum at 28° C. The range of H-ion concentration of medium for its fair growth is rather wide.

Successive periods of rainfall, high humidity, and temperatures around 28° C. are prerequisites for serious outbreak of the disease. Dry weather suppresses its development and results in the recuperation of plants not severely injured.

The fungus may live over winter on dead, diseased stalks in the field and infect cotton the following season.

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PHYTOPATHOLOGICAL NOTES

The Relative Susceptibility of Different Lots of Oat Varieties to Smut.[1]—In studies on physiologic specialization of the oat smuts at University Farm, St. Paul, one of the major difficulties inherent in the work has been the varying intensity of smut attack in successive years. In an earlier paper[2] consideration was given to the effect of varying environment in modifying the pathogenicity of purified collections of smut, and it was suggested that environmental factors might explain the variation. However, in 1940, field tests on the smut susceptibility of seed lots of the same oat variety suggested that plants grown from different seed lots might vary greatly in their response to smut and that much of the variation previously thought due to different environments during the infection period may, indeed, have been attributable to this source.

TABLE 1.—*The amount of smut produced on different lots of Anthony oats by collection 96 of Ustilago levis*

Year seed produced	Place of seed production and percentage smutted heads			
	St. Paul	Waseca	Crookston	Duluth
1935	46	......	......	......
1936	72	......	45	......
1938	38	54	43	42
1939	14	43	37	22

Eleven seed lots of Anthony oats were obtained through the courtesy of H. K. Wilson, Division of Agronomy and Plant Genetics, University Farm, St. Paul, Minnesota. These seed lots varied in that some were grown at University Farm in the years 1935 to 1939, while others were grown at the branch stations of the University of Minnesota at Waseca, Crookston, and Duluth. The source of seed and the year when grown are given in table 1.

The seed was surface-sterilized in 1 : 1000 mercuric chloride, washed in running water for 2 hours, and then dried. The seed was inoculated by means of the partial-vacuum method, using a chlamydospore suspension of 0.5 g. spores per 1,000 cc. of sterile water. Five g. of seed were planted for each 8-ft. row and each seed lot was replicated 5 times. A purified collection of *Ustilago levis,* collection 96[2] was used. Infection counts were based on the percentage of smutted heads.

The results obtained indicate that there are differences in the susceptibility of plants grown from different seed lots of one variety of oats. The range in infection varies from a mean of 14 per cent for the University Farm seed lot grown in 1939 to 72 per cent for the University Farm seed lot grown in 1936. Analysis of variance indicated that significant differences existed for the response of seed lots to smut attack. The 1939 seed lot from Duluth also gave rise to a low number of smutted heads, and the Waseca and Crookston 1939 seed lots were also lower than the seed lots for the previous year, although the differences in the two latter cases do not reach the level of significance.

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[1] Paper No. 1884 of the Scientific Journal Series, Minnesota Agr. Exp. Station. Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 65–1–71–140, sponsored by the University of Minnesota. 1940.

[2] Tervet, Ian W. Problems in the determination of physiologic races of *Ustilago avenae* and *U. levis.* Phytopath. 30: 900–913. 1940.

A full explanation for these differences in susceptibility has not yet been obtained. However, it is unlikely that the variation was due to genetic differences in the seed lots, for the seed for each year's crop was obtained from selected plots at University Farm and then distributed to the branch stations. There is a correlation between rapidity of germination, with its corollary of a decreased time for infection, and resistance to smut. For instance, greenhouse tests showed that in about 80 per cent of the University Farm 1939 seedlings the first appeared in 6 days, while the 1935 and 1938 seed lots were approximately 24 hours slower. The 1936 seed lot was still slower, requiring 8 days before most of the seedlings had produced their primary leaf.

These data confirm the results of Lods and Coulson[3] who stated, in 1929, that seedlings derived from light seed of oats were more susceptible to infection by the oat smuts than seedlings from large seed. Also, Holton and Heald[4] showed that seed lots of Marquis wheat, grown in different localities, gave rise to plants that varied in their reaction to bunt. Thus it appears that the conditions under which a crop is grown may influence the reaction of the succeeding crop to the seedling-inoculated smuts.

Other tests were run with seed lots of Rusota, Iogold, and Gopher and similar results obtained. Also, parallel experiments, using collection 27 of *Ustilago avenae*,[5] gave like results, although the amount of smut produced by this collection was less than with *U. levis*.—IAN W. TERVET, Division of Plant Pathology and Botany, University Farm, St. Paul, Minnesota.

Rhizoctonia Root Rot of Sweet Clover.[1]—A root rot of sweet clover caused by *Rhizoctonia* has been found in several widely separated districts of Manitoba, Canada, and in the State of Minnesota, U. S. A. Up to the present time, apparently, *Rhizoctonia* has not been reported as pathogenic on sweet clover.

The fungus isolated from diseased sweet-clover roots is probably a strain of *Rhizoctonia solani* Kühn. The mycelium is typically that of *R. solani*, except that in young cultures it is somewhat lighter in color and produces an abundance of cottony aerial growth. The organism is a very rapid grower, covering a Petri plate at temperatures of from 25–30° C. in less than 48 hours. At ordinary room temperature it forms brown, irregular sclerotia, but not until after a culture is from 2 to 3 weeks old. It is equally pathogenic on several varieties[2] of *Melilotus alba*, *M. officinalis*, and *M. suaveolens*, as well as on *Trifolium pratense*, *T. hybridum*, *Medicago sativa*, *Pisum sativum*, *Glycine max*, and *Vicia angustifolia*. It is not pathogenic, however, on any of the cereals. On sweet clover it causes a typical damping-off in seedlings, while in plants from 2 to 4 months old it attacks the roots, particularly near ground level, causing a dark-brown dry rot. Such plants become stunted, their lower branches wilt and wither, and, eventually, the whole plant may be killed.

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[3] Lods, E. A., and J. G. Coulson. A preliminary report on size of seed in relation to smut infection in oats. Twenty-first Ann. Rept. Quebec Soc. Protect. Plants (1928–1929): 80–85. 1929.

[4] Holton, C. S., and F. D. Heald. Studies on the control and other aspects of bunt of wheat. Wash. Agr. Exp. Stat. Bull. 339. 1936.

[5] See footnote 2.

[1] Contribution No. 658, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa.

Judged by its virulence under experimental conditions both in the greenhouse[3] and in the field, and by its distribution, as indicated by isolations from diseased specimens obtained from various districts of Manitoba, the disease is evidently of considerable importance. In greenhouse experiments of the last 3 years, the fungus invariably caused from 90 to 100 per cent damping-off in sweet-clover seedlings, and killed from 50 to 85 per cent of the older plants. Field results of the first 2 years of the investigation were inconclusive owing to the failure of the sweet-clover seed to germinate because of drought. In the spring of 1940, however, sweet-clover seedlings in the inoculated rows were killed 100 per cent. Plants of the two varieties, Common Yellow and White Blossom, inoculated after 6 weeks of growth, were reduced in yield of forage by 47.7 and 32.5 per cent, respectively. Isolations from diseased sweet-clover roots show that, in the earlier stages of root-rot development, the organism most commonly present is *Rhizoctonia*, while, in the more advanced stages, species of *Fusarium* predominate—a fact indicating that, at least under certain conditions, *Rhizoctonia* is primarily responsible for the disease.—W. J. CHEREWICK, Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba.

"Bends," a New Disease of Grasses and Cereals.[1]—A disease of grasses and cereals has been observed in recent years in the Pacific Northwest that, apparently, is quite unlike any heretofore described. The symptoms are characteristic regardless of the species affected. At, or sometimes above, the uppermost node a more or less sharp but not angular bend in the culm occurs. Very often the bend is complete, so that the portion of the culm beyond the bend hangs approximately parallel to the portion below it (Fig. 1). The bent region is usually more or less discolored, especially on the

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[2] The following varieties of sweet clover were tested both as seedlings and older plants—White Blossom, Erector, Waseca White, Iowa White, Madrid White, Common Yellow, Aura Yellow, Madrid Yellow, Albotrea, Alpha No. 1, Alpha No. 2, Minnesota Yellow, Zouave, Grundy, Ohio Evergreen, Redfield Yellow, and Redfield No. 40937.

[3] Studies made mostly in the Department of Plant Pathology, University of Minnesota.

[1] Grass-disease investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Soil Conservation Service, Division of Nurseries, U. S. Department of Agriculture, and the Divisions of Plant Pathology and Agronomy of the Washington Agricultural Experiment Station, Pullman, Washington. Published as scientific paper, No. 475, College of Agriculture and Agricultural Experiment Station, The State College of Washington.

upper side, and is brittle. Usually the inflorescence is more or less normal, and even matures seed.

The disease was first noticed in June, 1937, on *Agropyron sibiricum* (Willd.) Beauv., when the writer, with D. C. Smith, was taking disease notes in the observational row nursery of the Soil Conservation Service at Pullman, Washington. It was next found, the same season, in the Bureau of Plant Industry grass-smut nursery on crested wheatgrass and various *Bromus* spp., and still later, on Albit wheat. In the next 3 seasons the malady appeared increasingly prevalent. At the close of the 1940 growing season 37 species, in 16 genera, had been found affected with this peculiar disease, as follows: *Agropyron caninum* (L.) Beauv., *A. cristatum* (L.) Gaertn., *A. griffithsii* Scribn. and Smith, *A. inerme* (Scribn. and Smith) Rydb., *A. sibiricum* (Willd.) Beauv., *A. spicatum* (Pursh) Scribn. and Smith, *A. subsecundum* (Link) Hitchc., *A. trichophorum* (Link) Richt.; *Andropogon hallii* Hack.; *Bromus brachystachys* Hornung, *B. brizaeformis* Fisch. and Mey.,[2] *B. ciliatus* L., *B. commutatus* Schrad., *B. inermis* Leyss., *B. kalmii* A. Gray, *B. marginatus* Nees, *B. pacificus* Shear, *B. secalinus* L., *B. tectorum* L., *B. vulgaris* (Hook.) Shear; *Dactylis glomerata* L.; *Distichlis stricta* (Torr.) Rydb.; *Elymus condensatus* Presl., *E. glaucus* Buck., *Eragrostis lugens* Nees; *Festuca elatior* var. *arundinacea*[2] (Schreb.) Wimm., *F. idahoensis* Elmer, *F. rubra* L.; *Hordeum brevisubulatum* (Trin.) Link; *Hyparrhenia hirta* (L.) Stapf; *Koeleria cristata* (L.) Pers.; *Melica torreyana* Scribn.; *Muhlenbergia racemosa* (Michx.) B. S. P.; *Secale cereale*

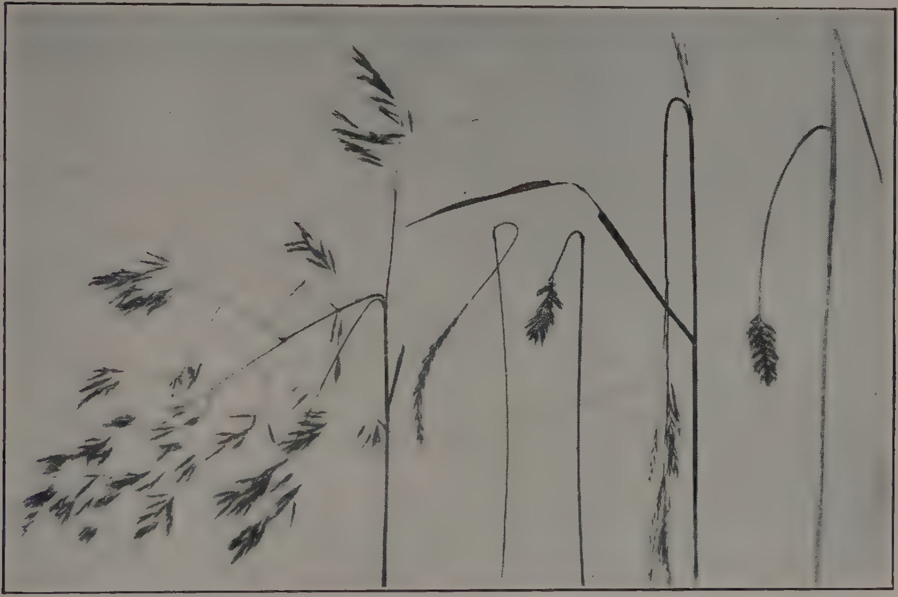

FIG. 1. "Bends," of *Bromus tectorum* (left), *Agropyron sibiricum, A. cristatum, B. pacificus,* and *Triticum aestivum.*

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[2] Reported from Ann Arbor, Michigan, by John R. Hardison in correspondence.

L. × *S. montanum* Guss.[3]; *Setaria lutescens* (Wiegel) F. T. Hubb.[2]; *Sporobolus asper* (Michx.) Kunth; *Triticum aestivum* L. (agron. var. Albit).

Incidence of the disease on such a wide range of hosts, and the marked uniformity of the symptoms produced on all these hosts lends some support to an hypothesis that it is of a parasitic nature. On the assumption that the parasite would be localized in the region of the bend, surface-sterilized bent regions and adjacent areas from 16 species of affected grasses were plated out on nutrient agar. Corresponding areas on apparently healthy plants were similarly treated. Two hundred and thirty-eight cultures of such fungi as *Alternaria, Chaetomium, Epicoccum, Helminthosporium, Hormodendrum, Macrosporium, Selenophoma,* and a few unidentified forms have been obtained. With two exceptions, nothing grew from the surface-sterilized portions of healthy culms. The variety of fungi isolated from the bent and adjacent regions is rather disconcerting, unless it is considered that the same symptoms could be caused by more than one fungous parasite, or, more probably, that most of these represent secondary invaders. On the other hand, none of the various fungi isolated may prove to be the cause of this peculiar disease. At the present time it must be admitted that the cause is quite unknown.

Various names have suggested themselves as being descriptive of this new disease of grasses and cereals, such as "hairpin disease," crook-neck, or "bends." The last name seems preferable because there is always more or less of a bend, but this is not always sufficient to resemble a hairpin or crook-neck, or the bend may be in excess of anything described by the last names mentioned. These variations are shown in figure 1.

Although many species of grasses have been found affected with "bends," it has not yet appeared sufficiently prevalent to be of economic importance. The disease does, however, challenge investigation—at least for purely scientific reasons.—GEORGE W. FISCHER, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Pullman, Washington.

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[3] Cross by D. C. Smith, Bureau of Plant Industry.

BOOK REVIEW

Transeau, E. N., H. C. Sampson, and L. H. Tiffany. *Textbook of Botany.* 812 pp., 424 fig. and 4 color plates. Harper and Bros. publishers, New York and London, 1940. $4.00.

This is indeed a *new* textbook of botany. As the domination of mycology in phytopathological textbooks has been replaced by a much broader treatment of the science of plant diseases, so have the authors of this new text removed the last vestiges of phylogenetic or anatomical domination from the beginner's text in botany. In this new work the various artificial branches of plant science are presented in proper perspective. The complex interrelations of plants with the other elements of their environment are clearly presented. Most gratifying of all, the importance of the various aspects of plant life to man are constantly stressed in unique and effective ways. The book should bring about not only a stimulation in interest concerning plants but will undoubtedly serve as an important factor in developing a ''conservation consciousness'' on the part of present day students. The teacher of elementary botany usually presents his subject from both the *cultural* and *technical* viewpoints. Needs of botanical specialists must be supplied without confusing the general student with too many technical details. The present work admirably supplies the needs. The authors intend the book to be used as a *supplement* to what is observed and discussed in the laboratory, field, and classroom. The many excellent diagrams, drawings, and photographs (including color plates) are well chosen and add much to the general clarity and utility of the book. Selected references are appended at the end of each chapter as a guide to students who wish to go further into the subjects discussed. The organization of material is such that the instructor can assign the various chapters in varying sequences based on local conditions.

There are 53 chapters. The first 6 chapters deal with aspects of plants more or less familiar to everyone, such as the general parts of plants, seasonal aspects of plants, local plant communities, points of view in the interpretation of plant behavior, etc. In these first chapters the student is led in a logical and fascinating way to the more fundamental aspects of plant life, which follow. Chapter 7 deals with cells as biological units, and chapter 8 with the tissue system of leaves. With the information of these two chapters as a background, the student can then appreciate ensuing chapters on ''environment and leaf development,'' ''hereditary differences in leaves,'' food chemistry of plants (5 chapters) and the uses of food in plants (3 chapters dealing with respiration, development, and substances made from foods). Chapters 20 to 25 deal with biological relations of green plants, interrelations of the parts of a plant, processes involved in the entrance and movement of materials within the plant, transpiration and its effects upon plant development and distribution. Three chapters are devoted to forms and processes in stems, two on roots, and four on the floral reproduction in plants including growth, dormancy, and germination of seeds. Chapter 35 deals with the vegetative multiplication of flowering plants. Some of the other subjects dealt with in later chapters are heredity and associated phenomena, underwater environments of plants, plants of the past, and the vegetation of North America. Ample coverage is given the bacteria, fungi, algae, bryophytes, pteridophytes, and seed plants. Chapter 45, which deals with phytopathology, is remarkably comprehensive and cannot fail to give an appreciation of this important branch of plant science.

While the text is open to negative criticism in minor points, these do not appreciably detract from its usefulness. The reviewer found the book interesting reading. In his opinion it cannot fail to serve as a potent catalyst in the development of a new generation of well-trained and enthusiastic scientists. Furthermore, general students who are guided in their study of plants by this text will have a keener appreciation of life.—Mark W. Woods, Division of Plant Pathology, Department of Botany, University of Maryland, College Park, Maryland.

CHEMICAL INACTIVATION AND THE REACTIVATION OF A PLANT VIRUS

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There is much uncertainty as to the nature of the change associated with the loss of infectivity of viruses that results from various experimental treatments to which they may be subjected. Loss of infectivity may be an indication of the death of the virus or it may be only a temporary loss of activity (inactivation), which, under proper treatment, may be regained (reactivation). In some plant-virus studies, the theory has also been proposed and accepted that certain chemicals or substances, *e.g.*, trypsin, may render the host plant less susceptible to or even temporarily immune from virus infection, and that the loss of virus infectivity on contact with such substances is not to be interpreted as a direct effect upon the virus. In other instances, the problem may be complicated by specific host responses that may be induced by the virus itself and may be regarded as modified host susceptibility or as the action of specific anti-bodies on the virus. The latter subject is beyond the scope of the present study, although its results may have some bearing on interpretations in the immunological field of research.

The ability of a virus to become reactivated when the inactivating or inhibiting agent or factor is wholly or partly removed appears to be the most satisfactory clue to the nature of the response of the virus to deleterious agents. Reactivation of inactivated plant and animal viruses and of bacteriophages (11) has been accomplished previously in several instances with certain substances, but relatively little is known about this response of viruses to a variety of chemicals. The results here presented were derived from tests with a number of chemical substances on a single virus. They appear to justify some further interpretation of the response of viruses to lethal as contrasted with inactivating agents, and of virus reactivation as compared to host inhibition of virus infectivity.

Some conclusions representing a part of this investigation have been published earlier as abstracts (8, 9).

REVIEW OF LITERATURE

Much of the earlier literature on the influence of chemicals on viruses does not bear directly on the present problem. It may suffice to say that the relatively high tolerance of the virus of ordinary tobacco mosaic (tobacco virus 1) to such antiseptics as mercuric chloride, copper sulphate, Formalin, etc., at concentrations that are highly deleterious to microorganisms as well as to higher forms of life has long been known. On the other hand, this virus is readily inactivated by normally inocuous substances, such as plant or animal charcoal and the juices of *Phytolacca*. This inactivation has been attributed to virus adsorption by the materials in question. However, the behavior of tobacco virus 1 is not characteristic of plant viruses in general, and most plant viruses are far less tolerant to the typical deleterious chemicals (1).

Stanley's chemical studies on the virus of tobacco mosaic are of particular interest in relation to the more recent trends of study. His first paper (13) dealt with the effects of trypsin because of its already known inactivating power and the possible relation of proteolytic enzymes to the protein nature of viruses. He found, however, that the action of trypsin on the virus was immediate and, therefore, the loss of infectivity could not be regarded as of an enzymic nature. He concluded from differential-host behavior and dilution tests that the effect of trypsin in causing a loss in virus infectivity is due chiefly to its action on the host plant. In a later paper (15) Stanley reported the effects of 110 chemicals on virus infectivity, finding many had no effect, others increased infectivity, and still others caused a marked decrease in infectivity. In this paper he again emphasized the necessity of distinguishing the action of the chemical on the virus from its effect on the host. He classified the chemicals that have a direct inactivating action on the virus as protein-precipitating agents, oxidizing agents, and agents causing a hydrogen-ion concentration known to inactivate the virus. In a third paper (14) Stanley discusses the rates of inactivation at different hydrogen-ion concentrations. With the method used the rate of inactivation was found to be negligible between pH 3 and pH 8, fairly rapid between pH 1.5 and 2.5 and pH 9 and 10, and very rapid between pH 0.5 and 1.5 and pH 11.0 and 12.0. Thornberry (16) and others have demonstrated a similar relationship.

The data and the conclusions presented by Stanley in these papers suggested the need of further investigations, particularly with regard to the action of the chemicals on the susceptibility or predisposition of the host plant to infection. The interpretation of a host response to chemicals has been accepted or supported by several other investigators, as is illustrated by Thornberry's (17) and Chester's (3) work and more recently by Black's paper on inhibition of virus activity by insect juices. Went (18), working in our laboratory, first undertook to differentiate between the influence of chemicals on the virus and on the host, using copper sulphate and mercuric chloride. She concluded that the inhibitory action of these chemicals was attributable to the inactivation of the virus itself, that subsequent reactivation of the virus by dilution and dialysis is possible, and that the inhibition of infectivity with the chemicals she employed was not a host response. Ross and Stanley (12) later concluded that the action of formaldehyde is on the virus itself. Other writers also have disagreed with Stanley's conclusions with respect to trypsin.

It appeared that there might be some reason to distinguish between results obtained with the molecularly simple chemicals and those obtained with complex organic compounds such as trypsin and with substances wherein the chemical identity of the inhibiting agent was unknown, as with *Phytolacca* juice.

EXPERIMENTAL METHOD

Various techniques may be used in studying the basic questions involved in the reaction of viruses to chemical agents. The problem differs in one important respect from that of purely chemical investigations, in that the measure of the reaction is the survival of an often extremely sensitive living substance of ultramicroscopic size. The results therefore do not lend themselves readily to interpretation on the basis of previously known facts.

The concentration of the chemical used and the time of exposure of the virus to the chemical are the first factors to be taken into account. These may be modified to suit the purposes of the investigation, but, in general, it is only when one or both of these factors are reduced to the minimum that will cause loss of infectivity of the virus that significant evidence of virus behavior may be secured. Sometimes the immediate inactivation of virus by a substance such as trypsin may be of special interest, or the prolonged survival of virus in concentrated solutions such as toluene may be of particular significance, but generally it is the gradual loss of infectivity that permits the most satisfactory interpretations of the results.

The chief special technique involved in the investigations here reported is the removal of part, or all of the chemical from the virus-chemical mixture after the desired exposure, or, if this removal is not complete, the introduction of controls that will compensate for the residue of chemical so as to afford reasonable comparison. The removal of the chemical from the mixture has been accomplished or attempted by others in a variety of ways, as by heat, dilution, digestion by enzymes, dialysis, precipitation, pH adjustment, ultra-filtration and ultra-centrifugation. All methods have certain limitations, especially when chemical substances of uncertain behavior and properties are involved or when the virus is very sensitive to momentary contact with the chemical.

The data here presented are based largely on what may be called the agar-diffusion method. This method depends upon the different rates of diffusion of the virus and of chemical substances in small agar discs into which tobacco virus 1 is incorporated. The agar discs are immersed in the chemical to be tested, and, after the desired exposure, the discs may or may not be extracted with distilled water, as desired. The virus diffuses only very slowly out of these discs, and the chemicals diffuse at widely different rates of speed both into and out of the discs. Consequently, different periods of exposure to the chemical substances and to extraction with water are necessary.

The agar-diffusion method possesses many advantages, being, first of all, very simple and easily carried out without special equipment. The discs may be made up to carry approximately any desired number of virus particles, with a view to ease of counting and statistical treatment of local-lesion data. Large numbers of the discs may be made up at one time and comparative uniformity in the number of particles in the discs over months of time may be insured by preserving the discs in full-strength toluene. The discs are easily prepared for inoculation by squeezing them through the same small squares of cheesecloth used for the inoculation, with further maceration on the cloth-pad if desired.

The discs may be made by preparing a 3 per cent solution of agar in water with a concentration of virus that is estimated to yield the desired number of virus particles to each disc. Particle numbers ranging from 200 to 800 per disc may be desired for different purposes, but, where actual lesion counts are to be made, it is advantageous to hold the virus particle numbers to around 400 per disc. For this purpose, a 1:20 dilution of a clear filtered or decanted virus extract may be approximately correct. Using small flasks, 25 cc. portions of hot agar are prepared with a large warm pipette, and 5 cc. of the virus extract is added to the agar, after which the mixture is rotated gently so as not to introduce air bubbles. The mixture is then poured into Petri dishes resting on a level table. As many poured plates as desired may, of course, be prepared at one time. It is important to have the agar sufficiently hot (about 80° C.) to obviate any solidification on addition of the virus solution, which, in the case of tobacco virus 1 also, may be warmed in a water bath to 50° C. without harm before it is added to the agar.

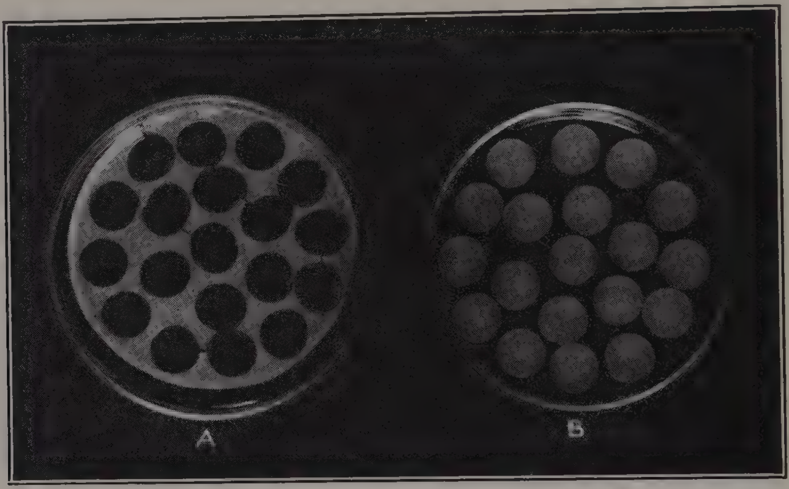

FIG. 1. Agar discs containing the virus of ordinary tobacco mosaic as used in the studies on virus inactivation and reactivation. A. A poured plate of agar from which the discs have been cut and removed. B. The agar discs as prepared for immersion in the chemical to be tested.

When the agar in the Petri dishes has solidified, they may be transferred to a refrigerator for a few days, if desired, before using. The discs should be cut when the agar is cold (about 10–15° C.) with a sharp cork-borer approximately 1.5 cm. in diameter (Fig. 1). A 9 cm. Petri dish will yield about 17 discs of this size. They may be removed easily and without injury with a spatula. If the discs are not to be used for several days, they should be transferred to a stoppered flask of concentrated toluene, from which they can be taken as desired. It is advisable to cover the discs with water for 2 to 12 hours before use in order to remove most of the retained toluene, which rises to the surface of the water. The removal of the toluene is only for the purpose of avoiding any possible effect it may have on the chemical added, since it does not affect the virus. Apparently no virus diffuses from the discs into concentrated toluene, and the toluene also prevents any loss of virus through microbial decomposition. After several weeks, enough water may be extracted from the discs by the toluene to cause shrinkage of the agar, but this may be remedied by covering the discs with water for a few hours before using them. Water removes some virus from the discs, but this loss is usually relatively small and is normally equalized in the tests by the comparative treatment of control discs.

The discs may be dropped into large-size test tubes (about 2.0 cm. in diameter) containing 5 cc. or more of the chemical substances to be tested, at any desired concentration, with discs in distilled water in other tubes for controls. Following the desired time of exposure, the inoculations to the host may be made without or following extractions. The extraction may be performed by several changes of distilled water over a period at least equal to the time of treatment but as much longer as may be found necessary. In our experiments we have generally made extractions with changes of water in a gently oscillating two-arm culture tube, a method that speeds up to some degree the rate of extraction. This apparatus is illustrated in an earlier paper from this laboratory (7, p. 1017). Extraction with running tap water is not satisfactory because the impurities of the water may introduce or form foreign compounds. Inoculations are conveniently made by placing 5 cc. of water in an evaporating dish in which is placed a square of cheesecloth approximately 3 inches wide. The treated disc is dropped onto the cheesecloth, squeezed through it with the hand, macerated, and mixed further with the water in the dish, and then wiped over the surface of a host yielding local lesions with tobacco virus 1. The hybrid *Nicotiana tabacum* × *N. glutinosa* was used in our tests; 6 of the oldest leaves on each plant were inoculated with each disc. Local lesions usually are produced in 3 days, and the 3 leaves showing the largest number of lesions are selected for counting. Where the number of lesions exceeded 200 and the results were widely different, the numbers were often estimated. Most of the tests were run in duplicate, and, where favorable host-plants were used, the results were commonly very consistent.

It became evident in certain trials, either because of the color of the disc or the non-infectivity of the virus, that the chemical used was incompletely removable from the treated discs by extraction with water. The results in such instances may usually be counter-balanced, in part at least, by employing control virus-free discs, treated identically, except that an amount of virus approximately equal to that in the virus discs is not added until just prior to inoculation. The virus-free discs are prepared in the same manner as the virus discs, except that water is used to replace virus extract. The chemicals are allowed to diffuse into the virus-free discs in the same way as into the others, and, when the virus is added subsequently, the immediate effect of the amount of chemical present may be measured. Such tests with chemical treatment of the discs both after and before the virus is added, together with comparable discs from which the chemical has been wholly or partly extracted with water, permit differentiations that have a bearing on the nature of the effect of the chemical on virus infectivity. The modified virus-free agar-disc method was, therefore, extensively used with the purpose of distinguishing between chemical inactivation of the virus itself and direct influence of the chemical on the host, which might be interpreted as reduced susceptibility to infection.

EXPERIMENTAL RESULTS

With the agar-disc method, other factors than concentration and time of exposure may influence the results; for instance, the possible reactions between agar and chemical and rate of diffusion of virus and chemical through the agar. Little is known about the influence of the low concentrations of the chemicals used on the relatively inert agar. Present information is based on the physical condition and color of the agar after treatment with the chemical and extraction with water. Most of the chemicals appeared to have no influence on the agar itself, although potassium permanganate, at some of the higher concentrations, destroyed the normal consistency of the agar discs, and a pH below 2.0 softened them to some degree. Lead acetate, on the other hand, hardened the agar. With ferric chloride and safranin the colors were fixed and not removable by extraction with water. With one exception it is believed that these modifications of the agar have not influenced the experimental results or conclusions drawn from them with respect to their influence on the virus. The exception referred to is that of *Phytolacca* extract. It is likely that this substance forms a permanent and insoluble combination with the agar. This interpretation, if valid, would limit the value of the results secured with this interesting substance by the agar-diffusion method.

It would be of special interest to make a more extensive study than has been done in the present investigation on the rate of diffusion of the virus from the agar discs into water, as well as the rate of diffusion of the virus into virus-free discs from surrounding extracts at different virus concentrations and different osmotic pressures.. It appears likely that this method of investigation would yield as reliable estimates of the size of the virus molecule by comparing it with known molecular structures and other substances as are obtainable by the application of relatively great external forces such as that involved in ultra-centrifugation. It is fairly clear from our preliminary trials that the virus moves out of the discs relatively rapidly the first day but gradually diminishes in rate and amount and may not be entirely removed with daily changes of distilled water, even after 20 days. The virus enters the agar from a virus extract relatively slowly the first day but the amount appears to increase steadily and would perhaps continue until an equilibrium of virus concentration is attained between the virus extract and the agar, a process that would perhaps require 20 days or more. In any case, the diffusion of most chemicals both into and out of the agar discs occurs at a relatively rapid rate, as may for example readily be observed when pure chemicals with natural color, *e.g.*, copper sulphate, are used. With the more complex substances, such as trypsin, the rate of diffusion may be very slow and in some instances, as with milk, it is, of course, not certain which particular component is the active agent on the virus.

The results from the agar-disc method of virus treatment may be illustrated by the data secured over a wide range of hydrogen-ion concentrations

TABLE 1.—*A representative list of chemical substances showing a range of hydrogen-ion concentrations used to treat agar discs containing tobacco virus 1, and illustrating the relation of pH and other factors to infectivity of the virus*

pH	Chemical substance	Concentration	Time treated		Lesions on 3 leaves from discs			
					In chemicals		Controls in water	
			Exposed	Extracted	Not extracted	Extracted	Not extracted	Extracted
		Per cent	*Hours*	*Hours*	*Number*	*Number*	*Number*	*Number*
1.2	Nitric acid	1.0	24	24	0	0	216	255
1.2	“	1.0	6	18	0	2	216	255
1.4	“	0.5	6	18	1	20	750	825
1.4	“	0.5	2	22	14	37	700	600
1.5	Hydrochloric acid	1.0	6	18	0	0	750	825
1.5	“	0.5	2	22	3	53	750	825
1.5	Oxalic acid	2.0	2	22	2	159	450	475
1.7	“	1.0	6	18	53	113	750	800
1.7	Bromine water	0.1	2	22	0	0	750	800
1.8	Citric acid	10.0	24	24	2	158	750	875
1.8	“	10.0	6	18	178	650	750	875
1.9	Picric acid	0.5	2	22	0	0	750	800
2.2	“	0.2	2	22	101	151	245	124
2.0	Ferric chloride	1.0	2	22	1	19	750	800
2.1	Lactic acid	5.0	24	24	5	182	650	550
2.4	Acetic acid	6.0	24	24	2	402	500	450
2.6	“	3.0	6	18	5	264	253	222
3.2	Tannic acid	5.0	2	22	0	31	245	124
4.0	Mercuric acetate	0.2	2	22	0	0	245	124
4.5	Copper sulphate	3.0	6	18	3	188	800	550
5.3	Phenol	5.0	6	18	1	0	253	222
6.6	Safranin	0.05	6	18	550	35	750	800
7.2	*Phytolacca* extract	1.0	48	24	1	0	202	236
8.5	Serum (horse)	1.0	48	48	0	176	130	296
9.7	Aluminum hydroxide	(sat. sol.)	6	18	650	650	750	800
10.8	Lithium carbonate	0.5	2	22	16	46	700	600
11.1	“	1.0	6	24	0	0	253	222
11.0	Trisodium phosphate	0.1	2	22	61	79	193	166
11.2	“	0.25	2	22	0	0	193	166
11.2	Potassium hydroxide	0.075	2	22	45	15	193	166
11.5	“	0.05	6	18	26	16	550	400
12.0	“	0.1	6	18	0	0	253	222

(determined by the glass electrode method), using different chemical substances (Table 1). The results, in general, agree with those of Stanley (14) and Thornberry (16) with respect to inactivation at the extremes of pH. Partial reactivation of inactivated virus at pH concentrations as low as 1.4 occurred, but none was obtained from virus inactivated at a pH above 11.0. From the results in table 1, however, it appears clear that the acids, bases, and salts give quite different responses at the critical extremes of hydrogen-ion concentration. Oxalic, citric, lactic, and acetic acids, for example, at similar inactivating pH concentrations, permit reactivation more readily than does nitric or hydrochloric acid. The strong inactivating action of bromine and of picric acid cannot be ascribed entirely to their relatively low hydrogen-ion concentrations; and the strong inactivating power of other substances shown in table 1 at hydrogen-ion concentrations between pH 3.0 and pH 10.0 must be explained on some such basis as oxidation, precipitation, or toxicity. The results with different chemicals taken altogether suggest that other factors than pH are also concerned with inactivation at the extremes of hydrogen-ion concentration used.

REACTIVATION OF THE VIRUS

Evidence of the reactivation of inactivated tobacco-mosaic virus (tobacco-mosaic virus 1) has been accumulating through the work of Stanley (13), Chester (4), Thornberry (17) and others, although it has retained a distinct element of uncertainty because of the belief that the host is in some way concerned with the results secured. In 1937, Went (18) presented evidence that the effect of copper sulphate and mercuric chloride was on the virus rather than on the host and that inactivated virus could be reactivated by dilution and by removal of the salts by dialysis. There is still doubt, however, as to the relative frequency of these phenomena and the merits of the two interpretations, namely, that of the inactivation of the virus itself as contrasted to the modification of host susceptibility by the chemical treatments applied.

A list of the chemical substances tested in the present experiments using the agar-disc method is given in table 2, along with a single typical response of the virus to each treatment. In the specific chemical concentrations and exposures shown it may be noted by comparing the "non-extracted" series with the comparable controls in water that a large proportion of the chemicals tested entered the agar disc in sufficient amounts to inactivate a considerable part, if not all, of the virus. However, when these chemicals were extracted with water from corresponding discs, a partial or complete reactivation of the virus resulted. Exceptions were bromine, hydrogen peroxide, iodine in potassium iodide, *Phytolacca* extract, potassium permanganate, safranin, trisodium phosphate, and potassium hydroxide. These chemicals consistently failed to yield any significant reactivation. The results with phenol, stannous chloride, and zinc sulphate are less certain, and it is possible that they do not all belong with the group that will not

TABLE 2.—*A list of chemical substances used to treat agar discs containing tobacco virus 1 with a typical example of the results secured at a single specific concentration, pH, time of exposure, and extraction with distilled water*

Chemical substance	Concentration	pH	Time treated		Lesions on 3 leaves from discs			
					In chemical		Control in water	
			Exposed	Extracted	Not extracted	Extracted	Not extracted	Extracted
	Per cent		*Hours*	*Hours*	*Number*	*Number*	*Number*	*Number*
Acetic acid	3.0	2.6	6	18	5	264	253	222
Aerobacter growth product	100.0	6.2	48	48	23	236	130	296
Alcohol	95.0	....	6	12	178	350	783	800
Aluminum chloride	1.0	3.5	24	24	1	14	202	187
Aluminum hydroxide	(sat. sol.)	9.7	6	18	650	650	750	800
Ammonium sulphate	25.0	4.6	6	18	81	675	550	550
Aspergillus growth product	100.0	7.9	48	48	44	160	130	296
Blood serum (horse)	100.0	8.5	48	48	0	176	130	296
Bromine (saturated in water)	0.1	1.7	2	22	0	0	750	800
Citric acid	10.0	1.8	6	18	178	650	750	825
Citrus fruit grapefruit	100.0	3.3	24	24	600	800	700	700
Citrus fruit lemon	100.0	2.3	24	24	226	800	700	700
Citrus fruit orange	100.0	3.4	24	24	600	800	700	700
Copper sulphate	3.0	4.5	24	24	5	418	800	550
Dextrose	10.0	....	4	16	167	216	241	350
Egg albumen (comm.)	10.0	....	4	16	135	187	241	350
Ferric chloride	1.0	2.0	2	22	1	19	750	800
Formalin (commercial)	10.0	5.2	6	18	14	68	550	400
Hydrochloric acid	1.0	1.5	2	22	3	53	750	825
Hydrogen peroxide	3.0	2.7	24	24	28	49	750	800
Iodine and KI	1.0	3.7	6	18	250	42	750	800
Lactic acid	5.0	2.1	24	24	5	182	650	550
Lead acetate	5.0	6.7	24	24	0	73	450	475
Lithium carbonate	0.5	10.8	2	22	16	46	700	600
Mannite	5.0	....	4	16	193	200	241	350
Mercuric acetate	0.2	4.0	2	22	0	0	245	124
Mercuric chloride	2.0	6.8	6	18	1	204	550	400
Milk	100.0	6.7	48	48	33	173	130	296
Nitric acid	0.5	1.4	6	18	1	20	750	825
Onion bulb extract	100.0	....	24	24	312	400	700	700
Oxalic acid	1.0	1.8	6	18	53	113	750	800
Phenol	5.0	5.3	6	18	1	0	253	222
Phytolacca leaf extract	1.0	7.2	48	24	1	0	202	236
Picric acid	0.2	2.2	2	22	101	151	750	800
Potassium dichromate	2.0	4.3	24	24	4	210	650	550
Potassium hydroxide	0.5	11.5	6	18	26	16	550	400
Potassium permanganate	0.05	7.5	4	4	3	7	162	193
Safranin	0.05	6.6	6	18	550	35	750	800
Silver nitrate	3.0	5.5	6	18	2	38	253	222
Stannous chloride	0.5	....	4	4	41	51	400	436
Tannic acid	5.0	3.2	2	22	0	31	245	124
Taka diastase	5.0	....	4	16	24	400	241	350
Trisodium phosphate	0.1	11.3	2	22	61	79	193	166
Tomato fruit extract	100.0	....	24	24	500	400	700	700
Trypsin	2.0	5.8	48	48	56	172	349	368
Urea	40.0	....	48	48	51	500	455	550
Zinc sulphate	2.0	5.8	6	18	425	200	550	550

permit reactivation. Twenty-eight out of 41 of the chemicals or substances tested which inactivated the virus also permitted complete or partial reactivation following extraction with water. Those that permitted reactivation included nitric, hydrochloric, and six organic acids, 10 inorganic salts, and alcohol and formalin; several of these are normally toxic, while 9 are not known to have toxic, oxidizing, or protein-precipitating effects, nor do they have an unfavorable hydrogen-ion concentration.

The reactivation of a virus following chemical inactivation at the proper concentration and time of exposure is, therefore, a relatively common phenomenon. It is also believed to be a highly significant one as regards the nature of the reaction between the virus and the chemical agent or substance. Perhaps it may be said from the data thus far secured that where the effect of the chemical is to oxidize the virus, reactivation is not possible. On the basis of this postulate, however, we must exclude potassium-dichromate from this category at the strengths and in the manner used in our experiments, although it is said by Stanley (15) to act as a virus-oxidizing agent. On the other hand, safranin, classified by Stanley as a protein-precipitant, has in our reactivation tests behaved more like the oxidizing agents.

If the mineral salts at the concentrations used are to be regarded only as virus precipitants, the results shown in table 2 are not easily interpreted. It seems necessary to include here a ''toxicity'' factor that may not yet be interpreted in strictly chemical terms in dealing with living organisms, from the bacteria up to the higher organisms, because of the great variability in susceptibility of species, and often of individuals, to such substances. Chemicals normally highly toxic to lower organisms usually will not permit reactivation when used at an effective concentration on the virus. On the other hand, chemicals regarded as efficient virus or protein-precipitants, such as ammonium sulphate, alcohol, and lead acetate, are not highly effective as virus inactivators. However, the factor of virus absorption or adsorption, which conceivably may be either physical or chemical in nature, must also be considered in the present connection. The behavior of the virus toward charcoal usually is accepted as an example of physical absorption, and it may be that the effect of *Phytolacca* extract on the virus is a more typical example of chemical adsorption. In view of the known peculiarities of adsorption phenomena in other fields of research, as well, it seems logical to consider the effects of trypsin and other substances, such as milk and blood serum, as belonging in this category. Stanley (15), however, accounts for the action of trypsin, and other substances that do not conform to the three chemical categories to which he ascribes inactivation as due to a modification of the host plant, rendering it less susceptible or, even immune, to infection by the virus. On this assumption, all inactivating chemicals at concentrations permitting reactivation may be regarded as capable of modifying host susceptibility, and this interpretation has essentially been accepted by several other investigators.

IMMEDIATE INACTIVATION OF THE VIRUS

Although it may require one or two minutes to mix a virus with the chemical to be tested and to inoculate the plant with this mixture, or a dilution of it, the results from such treatments are considered as "immediate" effects, even though the opportunity for continued influence of the chemical remains for a considerable additional period on the leaf surface after the inoculation is made, unless it is washed off. However, since the virus infection normally takes place almost immediately upon inoculation, or not at all, it appears logical to differentiate "immediate" inhibition or inactivation of a virus from inhibition or inactivation requiring a definitely longer period of time. Many toxic chemicals that inactivate the virus slowly at low concentrations may permanently inactivate or kill the virus immediately at higher concentrations. As may be expected, chemical concentrations that inactivate the virus slowly have little or no immediate effect on the virus (Table 3).

TABLE 3.—*The effect of certain chemicals added directly to tobacco virus 1 when the mixture is inoculated immediately to the host plant. Representative concentrations show that such chemicals are not all "immediate" inactivators*

Chemical	Concentration	pH	Lesions on 3 leaves with	
			Treated virus	Nontreated control
	Per cent		*Number*	*Number*
Acetic acid	4.0	2.5	75	500
Aluminum chloride	1.0	4.4	100	150
Bromine water	0.1	1.8	0	500
Copper sulphate	6.0	5.7	0	400
Ferric chloride	1.0	2.0	42	500
Formaldehyde	2.0	7.4	1	300
Hydrochloric acid	0.5	1.5	300	400
Hydrogen peroxide	3.0	2.7	15	400
Iodine and KI	1.0	3.7	20	400
Lead acetate	5.0	6.7	14	600
Lithium carbonate	0.5	10.8	600	600
Mercuric acetate	0.5	3.6	30	600
Mercuric chloride	2.0	6.8	0	400
Oxalic acid	2.0	1.5	4	500
Picric acid	0.5	1.9	76	600
Potassium dichromate	1.0	4.5	100	150
Potassium hydroxide	0.1	11.9	300	400
Potassium permanganate	1.0	....	8	161
Safranin	0.1	7.7	20	400
Silver nitrate	3.0	5.5	50	150

Of special interest in the present connection is the group of substances that are neither toxic, protein-precipitating, nor oxidizing agents; nor are they substances having extremes of pH that cause virus inactivation, even though they may induce complete loss of infectivity when mixed with the virus. Charcoal and *Phytolacca* juice were early examples of this group. More recently the behavior of trypsin and many other substances has stimulated new interest in this kind of virus inhibition. In studying the influence of microorganisms on tobacco virus 1 the writer found that the growth-products of *Aerobacter aerogenes* and *Aspergillus niger* were highly and immediately inhibitory to plant viruses (8) as contrasted to growth products from several other organisms. Common substances of animal origin, such as milk, blood or blood serum, and aphid extract are highly and immediately inhibitory to the virus (Table 4) and behave essentially as do the growth products of *Aerobacter*. Other substances tested were onion-bulb extract, which was not inhibitory; tomato fruit extract, mildly inhibitory; and lemon juice, markedly inhibitory. The inhibitory potency of these substances may be shown by the fact that when the virus of ordinary tobacco mosaic or certain other plant viruses in concentrated form are treated with an equal volume of 2 per cent trypsin solution, or with milk, blood serum, or the growth-products of *Aerobacter* or *Aspergillus,* there ensues an immediate and generally complete inhibition of infectivity. Similarly, dilutions up to 1:1000 of some of these substances, such as *Phytolacca* extract, retain much of the inhibiting action (Table 5). Stanley's host-modification theory of

TABLE 4.—*Comparing the inhibitory effect of a number of organic substances when added directly to tobacco virus 1 and inoculated at once to the host plant*

Substance tested	pH	Concentration	Lesions on 3 leaves: Treated virus	Lesions on 3 leaves: Nontreated control
		Per cent	*Number*	*Number*
Aerobacter growth product	6.2	100	1	550
Aspergillus growth product	7.9	100	1	200
Blood serum (horse)	8.5	100	0	500
Egg albumen (comm.)	......	10	8	161
Grapefruit	......	....	....	....
Insect (aphid) extract	......	....	0	161
Lemon fruit extract	2.3	100	28	465
Milk (cow's)	6.7	100	0	900
Onion bulb extract	......	100	500	465
Orange fruit extract	3.4	100	34	465
Phytolacca extract	7.2	10	0	600
Tomato fruit extract	......	100	167	465
Trypsin	5.8	2	16	600
Urea	......	20	350	400

TABLE 5.—*Illustrating effect of dilution upon the inhibiting effect of certain substances when added directly to tobacco virus 1*

Dilution of inhibitor	Lesions on 3 leaves: *Phytolacca* (extract)	Milk (cow's)	*Aerobacter* (product)	*Aspergillus* (product)	Serum (horse)	Trypsin
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
0	0	0	1	1	0	0
1:10	0	1	2	7	5	20
1:100	0	48	53	31	70	128
1:1,000	4	....	84	....	500	....
1:10,000	221	....	....	....	600	....
Water control	200	240	176	212	500	186

the inhibitory action of trypsin is based chiefly on the difference in reaction of the two host species used in the tests and on results from the addition of small amounts of trypsin to various dilutions of the virus, a treatment that caused a fairly constant decrease in the number of consequent lesions. The former result may be attributable also to natural differences in predisposition to infection, and the latter to adsorption phenomena leading to inactivation.

After attempting other methods of securing evidence on direct virus inactivation, as contrasted to host-modification, it was concluded that the "modified" agar-disc method, described in the foregoing, was quite as satisfactory as any now available. The results may be best illustrated by its application to the simpler chemical compounds, (Table 6). Each of the

TABLE 6.—*The inhibitory effect on tobacco virus 1 of comparable amounts of chemicals diffusing into agar discs, containing the virus before treatment, as contrasted to virus added to comparable discs after treatment.* (*Exposure 6 or 24 hours. Extraction 18 or 24 hours*)

Chemical concentration	pH	Virus added before or after exposure to chemical	Lesions on 3 leaves from discs			
			Not extracted with water		Extracted with water	
			In chemical	Control in water	In chemical	Control in water
			Number	*Number*	*Number*	*Number*
Acetic acid 3 per cent	2.6	Before	5	253	264	222
		After	89	119	97	103
		Before	3	202	106	187
		After	103	111	78	78
Aluminum chloride 1 per cent	3.5	Before	3	253	8	222
		After	175	119	172	103
		Before	1	202	14	187
		After	125	111	215	78
Copper sulphate 3 per cent	4.5	Before	0	506	516	740
		After	126	522	500	700
Formalin 10 per cent	5.2	Before	0	506	23	740
		After	212	522	700	700
Lithium carbonate 1 per cent	11.1	Before	0	253	0	222
		After	188	119	155	103
		Before	0	202	0	187
		After	183	111	218	78
Nitric acid 0.5 per cent	1.6	Before	74	506	21	500
		After	446	522	475	700
Phenol 5 per cent	5.3	Before	1	253	0	222
		After	182	119	56	103
		Before	2	202	0	187
		After	82	111	64	78
Potassium hydroxide 0.1 per cent	12.0	Before	0	253	0	222
		After	95	119	62	103
		Before	0	202	0	187
		After	43	111	104	78
Potassium hydroxide 0.05 per cent	11.5	Before	0	506	0	500
		After	550	522	600	700
Potassium permanganate .015 per cent	7.5	Before	0	506	23	740
		After	212	522	700	700

chemicals shown diffused into the discs in amounts sufficient to largely prevent infection from the virus in the discs *before* treatment. The discs to which the virus was not added until *after* treatment demonstrate, however, that sufficient amounts of the chemicals did not diffuse into the discs in the same length of time to result in a comparable loss of infectivity. The amount of chemical applied to the host by this method is approximately the same in the discs where the virus is added *after* treatment as in discs where it is present *before* treatment with the chemical. Where the proper chemical concentration and exposure are used, therefore, the data show that the amount of the chemical diffusing into the discs, although not sufficient to significantly inhibit host infection, is enough to inactivate almost completely the virus present in the discs. Hence, these chemicals under the conditions shown obviously inactivate the virus and do not significantly influence host susceptibility.

In applying the same modified agar-disc method of comparison to the more complex organic substances that are immediately inhibitory to the virus, some difficulties arise as a consequence of the slower rate of diffusion of the substances into and out of the agar. In table 7 are shown 6 such substances, which are very potent immediate virus inhibitors, together with the average relative amount of reduction of virus infectivity secured in agar discs treated and extracted with water over wide ranges of time. According to this summary, trypsin diffuses most slowly through agar, while *Phytolacca* extract diffuses most rapidly. The very low figure for *Phytolacca* is largely due, however, to the almost regular failure to secure reactivation after treatment with this substance rather than to slow diffusion. Complete inhibition of the virus has never been secured in trypsin-treated agar discs, whereas it is obtained occasionally with milk, blood serum, and the *Aerobacter* and *Aspergillus* growth-products and frequently with the *Phytolacca* leaf extract. The experiments with trypsin also are complicated somewhat by the "self-digestion" of trypsin on contamination with organisms in long exposures, with resultant loss of virus-inhibiting power, a loss

TABLE 7.—*A comparison of the inhibiting effect of six substances on tobacco virus 1 in agar discs. Several variations in time of exposure to substance and time of extraction with water are combined in averages shown*

Inhibitor	Trials made	Average lesions on 3 leaves from discs	
		Treated	Water controls
	Number	*Number*	*Number*
Trypsin	55	69	285
Aspergillus niger growth product	42	46	276
Aerobacter aerogenes " "	27	39	277
Milk (cow's)	56	36	284
Blood serum (horse)	27	31	390
Phytolacca plant extract	59	3	301

that allowed "natural" recovery of virus infectivity. This interference may be prevented in part, however, by the addition of a few drops of toluene to the trypsin under test, or, as was done in earlier experiments, by using aseptic technique. In table 8 are shown the results of fairly typical tests by the modified agar-disc method using trypsin, milk, serum, the *Aerobacter* growth product and *Phytolacca* extract. With the exception of the latter, which apparently belongs in a separate category, these substances are seen to behave like trypsin in respect to virus inhibition. It is also evident from this table that the inhibitory effect of the trypsin, milk, serum, and the *Aerobacter* growth-product taken up by the disc was quite as great when the virus was not added until *after* treatment with the substances as when the virus was present *before* treatment and exposure was continued for a long period of time. In this respect, the results may be seen to differ from those secured with the chemicals shown in table 6. When these inhibitors are removed to a sufficient degree by water extraction, a part of the virus again becomes active (Table 8). The degree of inhibition produced by these nontoxic substances appears to depend only on the amount of inhibitor present at the time of inoculation, and is not appreciably influenced by time of exposure. The amount of trypsin and milk that diffuses into the agar discs is relatively minute; and, although sufficient to inactivate a small amount of virus, it hardly suffices to induce immediate modification in susceptibility of the host when spread over a large surface of the plant. The substances in question appear rather to have a loose but specific affinity for the virus, resulting in its inactivation, and permitting reactivation when removed. The behavior of trypsin, milk, blood serum, and growth products

TABLE 8.—*The inhibitory effect on tobacco virus 1 of comparable amounts of substances diffusing into agar discs when the virus is in the discs before treatment as compared to virus added to the discs after treatment*

Inhibitor	Virus added before or after exposure to inhibitor	Lesions on 3 leaves from discs			
		Not extracted with water		Extracted with water	
		In inhibitor	Control in water	In inhibitor	Control in water
		Number	*Number*	*Number*	*Number*
Trypsin 2 per cent	Before	56	130	172	296
	After	18	260	340	500
Serum (horse)	Before	0	130	176	296
	After	5	260	260	500
Milk (cow's)	Before	17	290	49	600
	After	16	256	74	500
Aerobacter growth product	Before	23	130	236	296
	After	50	260	400	500
Phytolacca extract	Before	0	130	1	368
	After	0	260	5	233

of certain microorganisms toward the virus is apparently a form of adsorption. The data offer no reason to believe that this form of adsorption differs fundamentally from the inactivation caused by low or nontoxic concentrations of such other chemicals as permit reactivation to be brought about by removing the chemical inactivator from the virus, *e.g.*, it appears to be a loose and reversible molecular affinity in both cases. Exposure to toxic chemicals at higher concentrations (which may often, but not necessarily, result in protein-precipitation), or to oxidizing agents and certain extremes of hydrogen-ion concentrations, apparently results in a close and irreversible combination. Under such conditions, removal of the causal agent is either impossible or results in no virus reactivation.

INACTIVATION BY PHYTOLACCA EXTRACT

The existence of strong virus inactivators in the extracts of green plants is apparently relatively rare, though extensive tests on this subject are lacking. The extracts of several plant species apparently show a moderate inhibitory effect on virus infectivity. It is interesting to note that among the first plant extracts tested was that of *Phytolacca*, which has proved to be a powerful agent in this respect. Duggar and Armstrong (5) were led to make this test for the reason that *Phytolacca* could not be artificially infected with the ordinary tobacco-mosaic virus, although the species was obviously susceptible to a virosis, now known to have been induced probably by a cucumber-mosaic virus.

The extract of *Phytolacca rigida* has been used in a limited series of tests in the research here reported, and we have found that it will immediately and almost completely inactivate tobacco virus 1 (Table 4). The extract does not deteriorate on standing for long periods and may apparently be preserved indefinitely in toluene, although heating to 100° C. reduces its inhibitory action on infectivity, as does also filtration through fine filters. It may be diluted up to 1:1000 with water and still retain much of its inactivating power (Table 5). Dilutions of *Phytolacca* extract as high as 1:100 will diffuse into agar discs and wholly inactivate the virus. The virus, however, cannot be appreciably reactivated following long extraction with water. Further attempts at treating virus-free discs with the plant extract, followed by the addition of virus to the water-extracted discs just prior to inoculation, show rather conclusively that the inhibiting substance is not readily, if at all, removable from the agar by the diffusion method (Table 8). The nature of the inhibiting action of *Phytolacca* extract on virus infectivity is, therefore, more obscure than that of other substances tested by the agar-disc method. It seems most likely that the *Phytolacca* extract forms a water-insoluble, inactivating substance in or with the agar. This behavior does not, therefore, preclude the possibility that the action of *Phytolacca* extract on the virus is of a similar nature to that of other nontoxic substances (*i.e.*, adsorption), but suggests that the combination is less readily separated.

INACTIVATION BY MILK AND SERUM

Cow's milk inhibits virus infectivity to a remarkable degree, acting instantaneously and practically completely on full concentrations of virus treated with an equal part of milk. It is almost as effective at dilutions of 1:10, but is somewhat reduced at 1:100. Pasteurization, sterilization, or boiling did not destroy the inhibiting property appreciably; skimmed milk and whey were equally as effective as whole milk (Table 9). Evaporated milk and buttermilk, however, proved less potent.

TABLE 9.—*The immediate inhibiting action of milk in different forms and with various treatments on the infectivity of tobacco virus 1*

Milk (Form or treatment)	Lesions on 3 leaves	
	Treated	Control
	Number	*Number*
Whole	3	800
Skimmed	0	500
Pasteurized	0	500
Evaporated	40	225
Evaporated (dil. 1: 10)	185	225
Buttermilk	42	900
Whey	0	900
Boiled (5 minutes)	2	900
Sterilized 100° C. 15″	4	240
Undiluted	0	240
Diluted 1: 10	1	240
Diluted 1: 100	48	240

Milk diffuses readily into agar discs, resulting in virus inactivation, but the results from the succeeding extraction with water are more variable. It is clear, however, that at least part of the inactivating substance can be extracted with water and that its behavior, therefore, resembles more nearly that of trypsin than that of *Phytolacca* extract (Table 8). Additional studies by R. W. Fulton in this laboratory on the components of milk show that casein is the active agent concerned. Lactalbumen is also a good inactivator but is present in too low a concentration to play an important part.

The inactivating action of milk has been tested on several other plant viruses, including cucumber virus 1, the potato veinbanding virus, tobacco-etch virus, and the potato ring-spot virus. Some of these viruses of the cucumber, veinbanding, and etch type are even less tolerant to milk than tobacco virus 1, but potato ring-spot appears to be more tolerant than cucumber virus 1. A similar result was obtained with the potato ring-spot virus, using the *Aerobacter* inactivator. The results suggest the possible use of such a substance as milk in separating certain virus combinations (1).

The inactivating power of milk is of special interest and should be worthy of more detailed study in relation to certain human and animal viruses. It seems probable that such a substance may be a natural factor in the prevention of certain virus diseases, especially in early life. Evidence of other virucidal substances in human diseases has, for example, been recently reported by Jungeblut (10) and Francis (6).

Raw horse blood, or horse-blood serum, was used in several tests on tobacco virus 1 and was found to retain a considerable inactivating power up to dilutions of 1:100. Serum inactivation is immediate and complete at normal concentration and resembles that of milk in many respects. The serum inactivator diffuses more quickly into the agar discs than does the milk inactivator and is apparently more readily removed by water extraction than is either milk or trypsin, thus demonstrating reactivation of the virus more readily. It, therefore, differs in this respect very strikingly from Phytolacca extract (Table 10). The extensive investigations on sera and anti-sera in relation to both plant and animal viruses suggests that the agar-disc method of study might be helpful in separating some of the responses involved *in vitro*.

TABLE 10.—*Average number of lesions from five agar discs containing tobacco virus 1 exposed to various substances and extracted with water for comparative lengths of time, illustrating the relative extent of inactivation and of reactivation*

Treated with	Lesions on 3 leaves from discs	
	Treated but not extracted	Treated and extracted with water
	Number	*Number*
Phytolacca extract	2	3
Blood serum (horse)	5	61
Aspergillus growth product	48	79
Milk (cow's)	84	118
Trypsin 2 per cent	37	163
Aerobacter growth product	33	234
Water, distilled (control)	270	295

INACTIVATION BY GROWTH PRODUCTS OF MICROORGANISMS

In a recent paper from this laboratory (7) it was shown that microorganisms differ greatly in their respective rates of inactivating ordinary tobacco-mosaic virus. The most efficient was *Aerobacter aerogenes*. It, therefore, became of special interest to study further the behavior of this particular organism in relation to the virus.

Preliminary experiments showed that *Aerobacter aerogenes* could produce in culture a substance that was immediately inhibitory to virus infectivity, whereas the majority of other organisms studied in the previous contributions were apparently only capable of destroying the virus through the slow process of decomposition or digestion. The organisms used in this study were the same as those employed earlier (7). The routine methods of culture and inoculation also were essentially the same, except that the cultures usually were made in greater quantity in 500 cc. Erlenmeyer flasks instead of test tubes. The nutrient broth was made up to 3 g. beef extract, 10 g. peptone, 10 g. dextrose to 1000 cc. of water, at a pH of approximately 6.5. After the organism had grown in culture the desired length of time—normally, 10 days or more—the cultures usually were autoclaved for 20 minutes and stored in the refrigerator, so that comparable samples might be intermittently taken from the same stock culture for two weeks or more.

The behavior of this substance toward the virus of ordinary tobacco mosaic was much like that of trypsin, milk, and blood serum. The chemical nature of the *Aerobacter* growth-product being entirely obscure, the problems that presented themselves related chiefly to the properties of the substance and the nature of its inhibitory action.

No serious attempt has been made to determine the relation of the culture medium to the formation of the inactivator by *Aerobacter aerogenes.* It apparently is formed in any medium permitting some growth of the organism. It was formed, for example, in a medium of filtered plant extract alone, in synthetic broth and on potato agar. It was formed most rapidly and in greatest concentration, however, in nutrient broth. The bacteria themselves and the sediment in broth cultures evidently contain less of the inactivator than does the liquid medium; consequently, the inhibitor is of the nature of an external secretion. The substance is probably formed as soon as the organism starts to grow, but it does not reach high concentration until after 2 to 3 days' growth at room temperature. The maximum concentration may not be reached until 10–12 days of growth at room temperature. The substance does not appear to diminish in potency in pure cultures after long standing, *i.e.*, after having been kept in solution in the refrigerator for as long as 100 days. In the presence of contaminating organisms, it is slowly destroyed.

The inactivator is relatively heat-stable. Heating to 100° C. apparently has little injurious effect, but autoclaving at 115° C. for as long as 30 minutes results in appreciable destruction. Autoclaving for as long as 2½ hours at 115° C. did not completely eliminate the inhibiting action, and the inhibitory element is tolerant to prolonged freezing at $-5°$ C. The *Aerobacter* inhibitor in the normal concentration formed in broth culture is quite as potent as trypsin, milk, or serum. A normal concentrate will, for example, completely inactivate an equal quantity of fresh virus extract. If this inhibitor, however, be diluted to 1:100 or 1:1000, much of its potency will be lost. The concentration of the inhibitor may be increased to a considerable extent by evaporation of the culture medium. If evaporation proceeds to the dry state, the product will be about normally active in 0.1 per cent solution, and will remain active in this state for a year or more. Attempts at precipitating the inhibitor have shown that it will withstand high concentrations of alcohol and ammonium sulphate. Ether, chloroform, or mercuric chloride (1:1000) are not injurious, and it is apparently not adsorbed by charcoal.

All efforts to precipitate and purify the *Aerobacter* inactivator have failed. The precipitates secured with ammonium sulphate and alcohol have shown no more inactivating power than that shown in cultures evaporated to dryness. The difficulties experienced by many workers in attempts at purifying bacterial toxins discouraged more intensive efforts in this direction.

The *Aerobacter* inhibitor does not pass readily through bacteria-proof filters of the Mandler and Seitz type, one-half or more of the inhibiting power being lost in the filtrate. The inhibitor was found not to dialize through cellophane (sausage) membrane, when the membrane holding the inhibitor was placed under running water for 3 days.

The *Aerobacter* inhibitor is not toxic in the usual sense, and at high concentration permits the free growth of *Aerobacter* itself or other common bacteria or fungi. The inhibitor does not only affect tobacco virus 1, but will inactivate a number of other plant viruses as well, such as the viruses of cucumber mosaic, tobacco ring-spot, potato veinbanding, and potato ring-spot. These viruses are all usually considerably more sensitive to "toxic" concentrations of chemicals than is tobacco virus 1. The potato ring-spot virus is, on the other hand, quite as tolerant to the *Aerobacter* inactivator as is tobacco virus 1, although the ordinary cucumber-mosaic virus, the tobacco ring-spot virus, and the potato veinbanding virus are somewhat less tolerant to the inhibitor than the two former viruses.

It is certain from comparative tests that many common bacteria fail to produce an inhibitor of the type developed by *Aerobacter aerogenes,* although *B. ruber balticus* and others also produce inhibitors. Certain fungi are also capable of producing a similar inhibitor. *Aspergillus niger* has been shown to form a substance in nutrient broth culture quite as potent as that formed by *Aerobacter aerogenes.* It also appears, though less definitely, that growth-products of *Botrytis cinerea* behave in like manner. Fungi offer an advantage here, because of easy removal from the medium. Preliminary tests of the properties and behavior of the *Aspergillus* inactivator have shown them closely similar to those of the *Aerobacter* inactivator.

Stanley (13) showed that a preliminary treatment of the leaves with trypsin before the inoculation of the leaves with the virus caused a marked decrease in the number of lesions produced. The shorter the period between trypsin treatment and inoculation, the greater was the effect of the trypsin. When the trypsin was washed off its effect was greatly reduced. Inhibitory effects of trypsin sprayed on the host plants lasted one week after application. If, on the other hand, the virus was applied to the leaf first, the trypsin had little or no inhibitory effect on infection, unless applied within about 30 minutes after inoculation of the leaf with the virus.

The results secured in the present investigation with the *Aerobacter* inactivator were strikingly similar to those secured by Stanley with trypsin. The application of this inactivator before inoculation often completely prevented infection of the host for as long as a week. The *Aerobacter* inactivator was even more effective in reducing infection when applied following inoculation than was 2 per cent trypsin. Results of a similar type were secured with milk, serum, *Phytolacca* extract, etc. Experiments of this type can hardly be expected to have a significant bearing upon whether or not the inhibition of infectivity occurs through an effect on the virus or on the host, since, if the inhibitor has an immediate inhibitory effect and remains intact on the leaves, it is most likely that the result will be the same whether the inhibitor and the virus are applied separately or in combination with each other.

DISCUSSION

The results and conclusions presented in this paper should be considered in part as an effort to apply a new experimental technique to certain difficult virus problems. The agar-diffusion method appears to have some definite advantages for studies on inactivation and reactivation. It, therefore, may possibly be greatly improved or adapted to special purposes. An obvious need exists for a practical and simple method, capable of distinguishing between a modification of host susceptibility and direct effect on the virus itself. This is at least partially attained by the agar-disc diffusion method. The diffusion of a chemical into agar discs and its subsequent removal may be minutely regulated by varying the concentration of the chemical, the time of exposure, and the time of extraction with water. The virus may be introduced into the disc either before or after the chemical treatment for comparative purposes. A treatment at or near the minimum required for loss of infectivity of the virus-containing disc should also inhibit to a like degree virus added to macerated discs at the time of inoculation, provided the effect of the chemical be to modify the susceptibility of the host. This is not the case with many chemicals of the ''toxic'' group tested. The removal of the inactivating chemical with resulting restoration of infectivity, is clearly reactivation of inactivated virus. It is concluded, therefore, that good evidence has been established against any modification of the host by such chemicals. The results secured with other chemical substances, such as trypsin, milk, and serum, are less conclusive but point in the same direction. The data have yielded no evidence that cannot be explained on other grounds more logical than that the host is immediately modified in susceptibility by relatively minute amounts of these substances distributed over a large surface of the inoculated host.

It is believed that the status of the virus problem has reached the point where distinctions should be made between permanent and temporary inactivation. Permanent inactivation implies destruction or death of the virus. Some may argue that a virus is not definitely known to be a living substance and, therefore, is not subject to lethal agents. Others may maintain that a virus can never be said with certainty to be destroyed or killed, that is, it may be only inactivated, even though submitted to the most drastic forms of physical or chemical treatments. Without attempting to meet these contentions, it appears logical in the present state of knowledge to recognize that certain physical and chemical treatments of virus result in permanent or irreversible loss of infectivity or death, as far as can be determined by any test now available. On the other hand, it is obvious also that it is possible for a virus to lose temporarily its infectivity through contact with various chemicals and that infectivity may be partly or wholly recovered under appropriate circumstances. The term *inactivation* should be reserved for known or suspected phenomena of this type, in order that a distinction may be made between this phenomenon and that of the more logical, if not proven, death or destruction of the virus.

The present investigations have shown that the *inactivation* and *reactivation* of the ordinary tobacco-mosaic virus is possible with a variety of chemical substances when effective concentrations are used. Many of the same chemicals used in a sufficiently high concentration or over a longer period of time result in destruction or death of the virus because of their toxic properties. Another group of strong inactivators, non-toxic to the virus at high or normal concentrations, comprises milk, serum, trypsin, etc. The action of these materials is believed attributable to a form of loose physical or chemical union with the virus particles, a union differing little from that of the toxic group used at effective inactivating concentrations only. No attempt has been made to correlate the results of the present investigation with those relating to the antigenic properties of virus-immune sera, as investigated by Chester (4) and others in plant and animal viruses, although it seems probable that related responses are involved in such reactions.

SUMMARY

The influence of various chemicals on the infectivity of the ordinary tobacco-mosaic virus has been studied by means of an agar-disc method, which permits the diffusion of chemicals to the virus when contained in the agar and the subsequent complete or partial extraction of the chemical with distilled water.

The results are based largely on the use of chemicals at a concentration and time of exposure that inactivated the virus but permitted reactivation by removal of the inactivating agent with water. Many of these chemicals at higher concentrations or longer periods of exposure cause an irreversible loss of infectivity.

Forty-one chemical substances harmful to infectivity have been tested and of these 28 have permitted reactivation of the virus. Reactivation is possible following inactivation with many common salts and with acids at the proper concentrations, but was not secured following treatment with certain oxidizing agents, nor with bases at a pH above 11.0, nor at concentrations and exposures much above the minimum required for inactivation.

A number of common organic substances were found to be powerful immediate inactivators of viruses. These include milk, blood serum, citrus fruit extracts, and growth products of certain microorganisms, chiefly *Aerobacter aerogenes* and *Aspergillus niger*. These appear to belong in essentially the same category with respect to the nature of the inactivating power as does trypsin, aphid extract, *Phytolacca* extract and possibly charcoal.

No evidence was secured to indicate that trypsin or other substances tested modify the host plant, rendering it less susceptible to or immune from virus infection. The results as a whole suggest that trypsin and certain other substances inactivate the virus through the formation of a relatively loose specific molecular union (chemical adsorption or physical absorption), which may usually be broken up by removal of the inactivator with water, provided the nature or concentration of the chemical, or the time of exposure to the chemical is not such as to cause destruction of the virus.

It is believed that sufficient evidence is available to justify a distinction between the inactivation of a virus and its destruction or death. It is suggested that the term "inactivation" be reserved to express demonstrated or probable temporary loss of infectivity when reactivation may be induced or may be possible on the logical grounds that no known lethal agent has been applied. Chemicals that cause destruction or death of the virus above definite concentrations and times of exposure may be separated into toxic (often, but not necessarily, protein-precipitating) agents, oxidizing agents, and agents with a pH of about 1.5 or lower and about 11.0 or above.

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LITERATURE CITED

1. ALLINGTON, W. B. The separation of plant viruses by chemical means. Phytopath. 28: 902–918. 1938.
2. BLACK, L. M. Inhibition of virus activity by insect juices. Phytopath. 29: 321–327. 1939.
3. CHESTER, K. S. Specific quantitative neutralization of viruses of tobacco mosaic, tobacco ring-spot, and cucumber mosaic by immune sera. Phytopath. 24: 1180–1202. 1934.
4. ————. Liberation of neutralized virus and antibody from antiserum-virus precipitates. Phytopath. 26: 949–964. 1936.
5. DUGGAR, B. M. and J. K. ARMSTRONG. The effect of treating the virus of tobacco mosaic with the juices of various plants. Ann. Mo. Bot. Gard. 12: 359–366. 1925.
6. FRANCIS, T., JR. The inactivation of epidemic influenza virus by nasal secretions of human individuals. Science (n. s.) 91: 198–199. 1940.
7. JOHNSON, J. and I. A. HOGGAN. The inactivation of the ordinary tobacco mosaic virus by microorganisms. Phytopath. 27: 1014–1027. 1937.
8. ————. Plant virus inhibitors produced by microorganisms. Science (n. s.) 88: 552–553. 1938.
9. ————. Plant virus inhibitors. Proc. Int. Congr. Microbiol. (N. Y.) 3: 100. (Abstract) 1939.
10. JUNGEBLUT, C. W. Occurrence of poliocidal substances in tears, saliva, and cerebrospinal fluid of normal individuals. Proc. Soc. Exper. Biol. and Med. 32: 1534–1537. 1935.
11. KRUEGER, A. P. and D. M. BALDWIN. The reversible inactivation of bacteriophage by bichloride of mercury. Jour. Gen. Physiol. 17: 499–505. 1934.
12. ROSS, A. F. and W. M. STANLEY. The partial reactivation of formolized tobacco mosaic virus protein. Jour. Gen. Physiol. 22: 165–191. 1938.
13. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. 1. Some effects of trypsin. Phytopath. 24: 1055–1085. 1934.
14. ————. Chemical studies on the virus of tobacco mosaic. 3. Rates of inactivation at different hydrogen-ion concentrations. Phytopath. 25: 475–492. 1935.
15. ————. Chemical studies on the virus of tobacco mosaic. 4. Some effects of different chemical agents on infectivity. Phytopath. 25: 899–921. 1935.
16. THORNBERRY, H. H. Effect of phosphate buffers on infectivity of tobacco-mosaic virus. Phytopath. 25: 618–627. 1935.
17. ————. Effect of tannic acid on the infectivity of tobacco-mosaic virus. Phytopath. 25: 931–937. 1935.
18. WENT, J. C. The influence of various chemicals on the inactivation of tobacco virus 1. Phytopath. Zeitschr. 10: 480–489. 1937.

ACTINOMYCES IN POTATO TUBERS

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The ordinary scab of the potato is produced by the stimulus on the cork cambium of one or more organisms of a type intermediate between the fungi and bacteria. Many authors and investigators have referred to the pathogens as *Actinomyces scabies* (Thaxt.) Güssow.

It is not the intention of the writer to disinter all the evidence in favor of or against this naming other than to point out that Erwin Smith (13) listed both *Actinomyces* and *Streptothrix* as bacterial names to be avoided and that his *Chlamydothrix* (Migula) has met no favor.

Scabies as a species name originated with Thaxter, whose very incomplete description of an organism producing scab on potatoes was supplemented by Drechsler (4), working under Thaxter's direction and in collaboration with the U. S. Department of Agriculture, who examined 5 pathogenic cultures from the latter source. Of these 5 cultures, 4 agreed in morphological and cultural characters, but the fifth was unlike them in the spirals formed by aerial spore-bearing hyphae. All 5 cultures, based on pathogenicity, became *Actinomyces scabies.*

The writer would state that in the following paper any reference to "the scab Actinomyces" does not imply *Actinomyces scabies* in the limited sense in which that species has been defined, but in the broader sense of any organism that will stimulate cork cambium. The possibility of a number of species inducing this effect is not precluded, but this statement would depend on the definition of a "species" in the *Actinomyces* group. The writer is inclined to believe that only one real species is involved, but that its pathogenicity is variable.

The part taken by the stomata and lenticels in the infection of potato tubers to produce scab has been known since its discovery by Caspary in 1857 and has been since emphasized by the work of other pathologists, Fellows (5) and Jones (6).

The inoculations by Fellows, made from pure cultures on potatoes growing in sterilized soil, were to disclose the period in tuber development when infections produce the largest and most numerous scabs. The scab lesion is a growth phenomenon with a progress paralleling that of the tubers. Fellows, adhering strictly to infection through stomata and lenticels, attributed the spread of the true pathogen to the middle lamella of the cells under them. Infected cells walls stained a dark-brown, especially at the angles where 3 or more cells come together. *Actinomyces* mycelium observed inside other cells were pronounced nonpathogenic. These nonpathogenic types were found in corky cells (dead or dying before invaded) near the tuber surface. Fellows based his observations almost entirely on sections of fresh material. Unable to find causal organisms near brown tissues, he assumed that they must be working at some distance from the spot where lesions were evident. The nonpathogenic types, found in necrotic areas up to 5 mm. in diameter, were inside cells. The subepidermal layers (cork and cork cambium) were browned and stimulated. Large tubers had a much higher percentage of surface covered by scab than smaller ones, and scab areas were more abundant on bud ends of tubers, presumably because of thinner cork and more abundant lenticels.

Jones (7) attempted to explain the histogenesis of "deep" scabs by successive stimulations of the meristem to cell invasion by fungus strands. Elongated cells were produced for a time, and then, as the stimulus subsided, the meristem resumed the formation of a normal cork layer, which served as a barrier against the advance of the strands. This barrier might be again penetrated, the stimulus of the meristem resumed, and abnormal, elongated cells formed. A second barrier intervened to stop growth, with the possibility of its being penetrated and the formation of a third barrier. More than 3 cork barriers were never observed, even in very deep scabs.

The origin of deep scabs as an invasion of lenticel tissue by the scab organism is supported by both investigators, as it was by the writer (9, 10). The scab types—shallow, shallow-broad, etc.—of older authors may be explained along similar lines. The type of scabbing or russetting in recent years on land once infected with 100 per cent deep scab has raised doubts in the writer's mind as to the nature of this brown, russet, rough cork, which appeared on tubers the first season in which potato culture was resumed after an interruption of 19 years (11). Similar skin abnormalities on Dutch potatoes have been noted by Miss De Bruyn (2). This brown roughness of the tubers is clearly different from the definitely defined, although often coalescent, lesions seen in the ordinary deep scab. The tuber is covered by a brown layer of nearly uniform thickness, often cracked and furrowed, which would seem to result from a general infection of the periderm and not from isolated penetrations through lenticels.

These facts stimulated a reinvestigation of the infection and spread of the pathogenic *Actinomyces* in the stomata, lenticels, epidermis, and cork cambium.

METHODS

Two general methods are available for the study of potato periderm and its derivatives, as well as the pathological conditions induced by the scab organism:

1. The mycelium of these scab organisms accompanies its growth and progress by excreting a brown pigment that stains the cell walls of the tuber and osmoses into the adjacent cells. This characteristic makes the progress of the brown pigment-secreting varieties easy to trace by the following method. Removal of the skin of the young growing tuber is very easy, since the outer layers can be torn from the underlying starch parenchyma, the brown-wall layer being the cork parenchyma. These bits of skin were

mounted in 10 per cent glycerine drops on a slide; left uncovered, a few drops of the glycerine were added as the water evaporated. After 2–3 days 50 per cent glycerine was substituted, being cautiously added at the margin of the drop containing the bits of skin. After a week the drops were covered and allowed to stand for some months to clear.

2. Small tubers were imbedded in paraffin and sectioned, 8–10 μ thick. (a) Some non-stained sections, mounted in Canada balsam, were examined. (b) Some were stained in safranin-fast green. (c) Others were stained by Gram's method, the counter stain being fast green. (d) Others were stained with Orseillin BB and counterstained with anilin blue. None of these methods was successful in differentiating the fungus from the walls of the potato cells.

3. A modification of the Gram technic was considered the most hopeful procedure.[1] Jones' method for Gram-positive bacteria, presented in detail by Rawlins (12), was unsuccessful on tuber sections; the dye was entirely removed by the alcohol. The following method gave excellent results:

1. In strong (5 per cent) gentian violet-(crystal violet) anilin oil solution for 24 hours.
2. Wash in water.
3. In Gram's iodine solution for 24 hours.
4. Wash carefully in absolute alcohol until no color flows from sections.
5. Clear in xylol, mount in balsam. No counter stain.

OBSERVATIONS

I. On the tuber skin surface

The Green Mountain tubers were grown on land badly infested with the scab organism and on adjacent land producing largely clean tubers. Tubers, 0.5 to 1.0 cm. in diameter, were dotted thickly with stomata, especially at the stem end; over the equator and bud end, lenticels were forming, all lenticels developing under stomata.

The guard cells of the stomata, as they grow in size, become surrounded by epidermal cells that, in surface view, do not differ in size, form, or wall thickness from other epidermal cells. A low papilla is raised by growth from beneath and the top of this slight elevation is the stoma.

Infected lenticels show guard cells surrounded by other cells enlarged and elongated with walls thickened and browned. In figure 1, A, the epidermis is uppermost, but in figure 1, B, the drawings show tuber skin mounted upside down. The browned walls seemed to be wavy, but a magnification of (500–900 ×) shows them split, the apparent waviness being due to the winding and tortuous path of a filament of the scab fungus. The brown color was localized in the cell walls, although some might diffuse into the cytoplasm of cells which they enclosed, especially at the angles where several met.

Satisfactory photomicrographs of the lenticels are difficult to obtain be-

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[1] Stain Tech. **16**: 63–66. 1941.

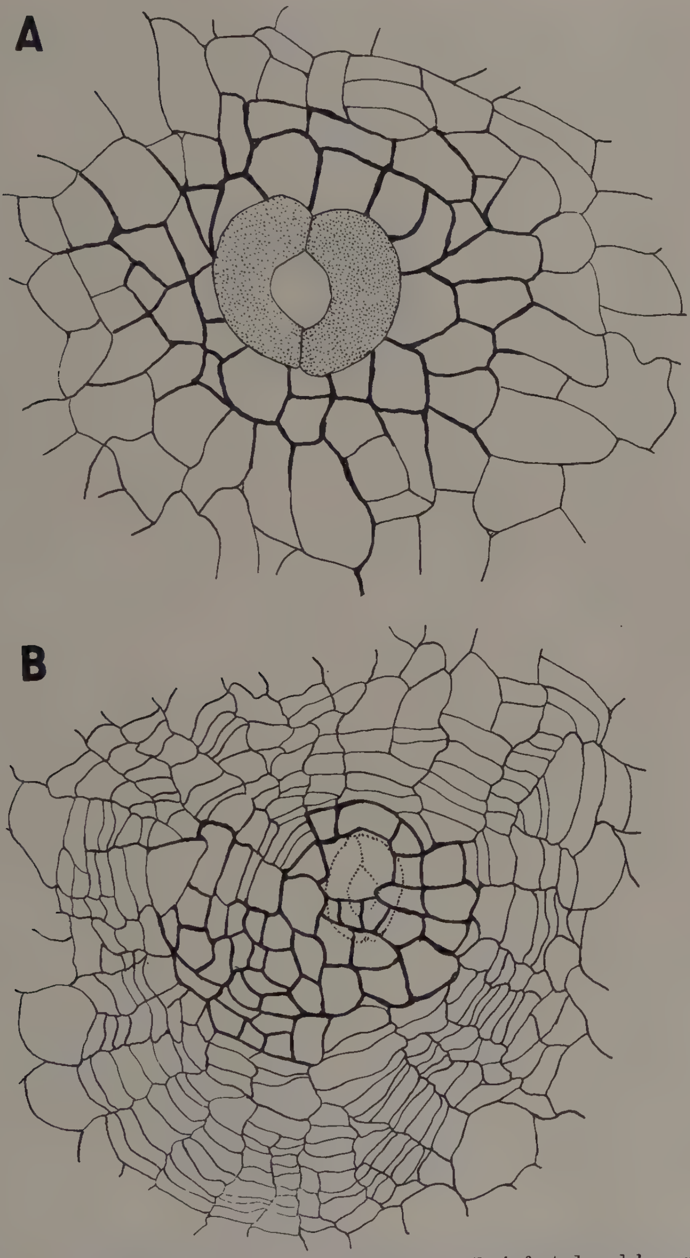

FIG. 1. A. Young lenticel and stoma with cell walls infected and browned. ×300. B. Young lenticel and stoma as seen in a piece of skin mounted upside down. Cells around stoma and cambium uninfected. ×120.

cause the papillae are so high. The mycelium cannot be seen in the dark-walled circle of host cells surrounding a young lenticel, but fine strands can be detected between the lighter adjacent cells. In general, they seem to follow between the cell walls in the middle lamellae, splitting the walls apart, but recognizable by their wavy appearance. Some strands appear to cross the cells, but these are located in walls parallel to the plane of the photograph. Fellows' (5) failure to observe mycelium may be explained on the theory that, in his sections, the winding, irregular mycelium would be cut into small bits and almost unrecognizable.

The other striking feature of young tuber skin, the dark-brown intercellular spaces, had been noted also by Fellows, who found, as around the lenticels, no trace of mycelium. He surmised, however, that here, as in lenticel infection, the mycelium worked at some distance from these areas. He observed numerous *Actinomyces* hyphae crossing the cells, but assumed them to be only saprophytes invading dead, or nearly dead, epidermal cells, since they appeared only on tubers grown in unsterilized soil. On tubers grown in sterilized soil and inoculated from pure cultures of his scab *Actinomyces* only stomata and lenticels were infected, and the epidermis was clear of all *Actinomyces* hyphae.

Some explanation must be offered, however, for the general scruffiness and browning of many tubers from land known to be heavily infested with parasitic strains that infect by way of lenticels. The stimulus is a uniform and general one, with a russetted-cork skin as a result.

Small tubers the size of a pea already show a patchy brownness, even under a hand lens. Such skin, mounted in glycerine (Fig. 2), illustrates the beginnings of brown areas. Isolated intercellular spaces are largest at angles, always dark-brown and filled with some jelly-like material, also brown. The arrangement and size of these areas have no relation to the stomata. Sometimes they are connected and sometimes isolated at the angle formed by 3–4 cells, even stringing out irregularly between a dozen cells. Straggling intercellular browning may rarely destroy patches of epidermal tissue.

This independence of brown patches and stomata is especially well shown in figure 2, D, where 19 brown, intercellular spaces are connected by thickened brown walls with a similar patch to the lower right. Around this diseased area are 3 normal stomata not connected with the infection.

A 100 × magnification of these bits of tissue does not permit a critical examination of the cell walls, but the margin of figure 2, D, magnified 500 ×, is seen in figure 2, C. The brown-walled cells themselves have not been much affected; certainly not more so than normal ones to the left. The cells with infected walls are slightly larger than those of the surrounding epidermis, and slightly raise the mass of tissue they form.

Brown walls and enlarged intercellular spaces are striking features of a surface view (Fig. 2, A and D). The walls seem enlarged and distorted, but this unusual appearance is due to the twisted strands of the mycelium. These strands secrete brown pigment, which tinges the host walls but does not percolate into the cells that the walls enclose. At the cell angles the pigment collects and tints the adjacent cells.

The injury induced by hyphae on the adjacent cells causes the formation

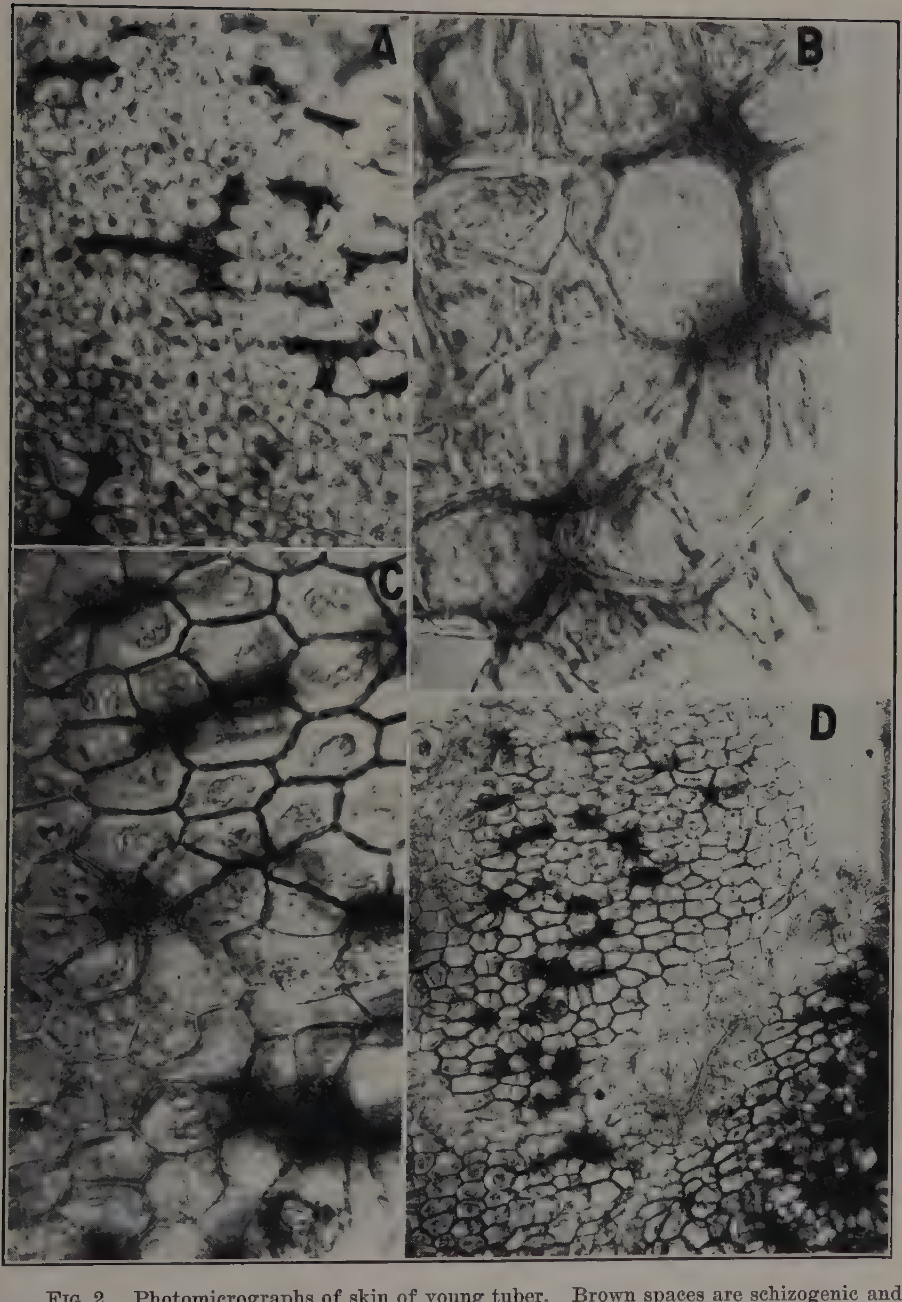

FIG. 2. Photomicrographs of skin of young tuber. Brown spaces are schizogenic and *Actinomyces* mycelium, radiate. B shows distribution of intercellular areas and thickened cell walls. C is part of D, twisting hyphae inside of browned cells walls. A and D, × 150; B, × 650; D, × 250.

of large, dark-brown, intercellular spaces. The dark jelly that fills these spaces makes observations on *Actinomyces* strands difficult, or almost impossible. Fellows' (5) failure to see them is quite excusable, not alone from the dark jelly, but also from the fact that the older bits of hyphae are empty and the walls are too transparent to stain. The *Actinomyces* filaments show at various spots, briefly in focus and then twisting out again, perhaps difficult to find in the reproduction, but well shown in photographic prints.

Filaments of considerable length (Fig. 2, B) emerge from the browned areas. If these filaments at times seem to cross inside cells, it is only an optical effect, since they are really imbedded inside the cell walls. The penetration of the cellulose walls is very doubtful; the microorganism is pectin- but not cellulose-dissolving. The active, living, working tips of the filaments are colorless; the secretion of this grown pigment seems to be one phase in the aging and destruction of the filaments themselves. Pure cultures on nutrient agar showed no color around the inoculation for some days after growth could be seen; later, the brown color began to diffuse out.

II. On Sections of Young Tubers

Sections of small white tubers, the size of a pea or less, are necessary to follow the development of the young stomata, the lenticels under them, and the spread of the hyphae.

The triple stain (gentian violet, safranin, and orange G) had been used on tuber sections made 20 years ago. Small tubers, cut longitudinally, showed numerous stomata (Fig. 3) cut across. Cork cambium and stomatal chambers had not yet been developed, but the stomata, without exception, had become infested with *Actinomyces* filaments. Cell walls had already been split and, in the chambers formed, the filaments had found food and lodging. The filaments retained the brilliant red of the safranin, while host walls were still faintly purple. Some filaments followed a cell wall, but near the stoma thickened intercellular spaces could be seen filled with *Actinomyces* threads.

Actinomyces filaments, usually Gram-positive in living portions, do not retain the Gram stain in older, dead filaments, Lieske (8). Making use of this Gram-positive property, mycelium in the tissues of young potato tubers was stained to determine the origin, nature, and depth of the infection, the means by which the filament made its way through the cells, and its effect on the host cells.

The filaments were not difficult to follow with a magnification of 500 diameters, provided the sections were correctly differentiated. Mycelial growth was limited to intercellular spaces, and had been seen in surface view, the cell walls having been split by dissolution of the pectin of middle lamellae. Split walls, always thicker than intact ones, owing to the intrusion of the *Actinomyces* filament, can be distinguished from them. The dark-blue stain of the filaments also mark them off in sections from the yellowish tint of the cell walls.

Filaments appear not to follow cell walls, but this is because of the thickness of the sections (8–10 μ); the filament may lie in a wall in the plane of the photograph.

Distribution of the Organism in the Cork Layer. For comparison, pieces of young tubers with white and others with brown skin were sectioned, stained, and compared.

White-skin tuber sections (Fig. 4) contain a general distribution of hyphae; but the intercellular splits are small and no large angular spaces appear at the corners of the cells. Some distortion of the cells may result, but not enough to raise the cork layer.

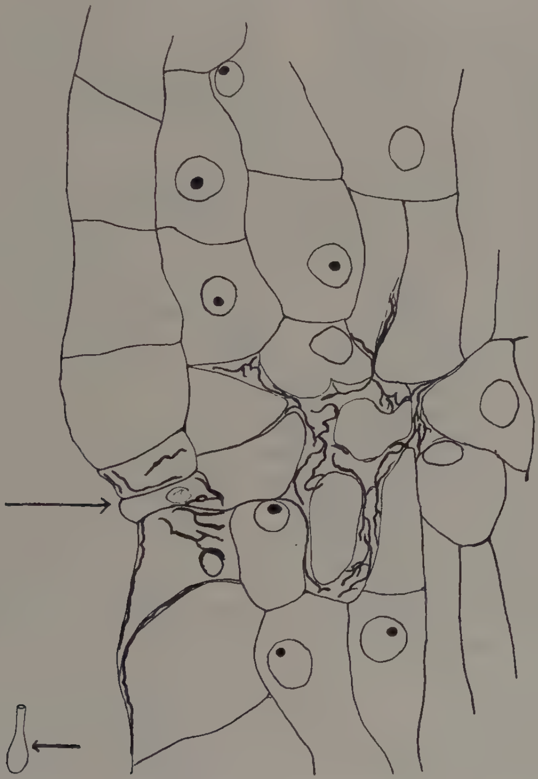

FIG. 3. Section of very young stoma, tuber at lower left; infection with *Actinomyces* hyphae. × 500.

Brown-skin tuber sections show a similar distribution of the hyphae, but large, schizogenic spaces filled with a dark-brown coloring matter lie at the base of the cork cambium (Fig. 4, B). The schizogenic spaces under the cork seem to be filled with brown excretion products and may extend to the surface of the cork as darkened areas. With a certain amount of differentiation, these products are as Gram-positive as the living organisms, an interesting sidelight on the theory of the Gram stain itself.

Shallow scabs seem to be the result of a general infection of the cork cambium by the brown-color-producing *Actinomyces*. The infection may occur at any cell juncture by the penetration of the parasite between the cells through the pectin of the middle lamellae. So far as can be observed, no cuticle covers the joints between the young epidermal cells, and any pectin-dissolving organism could enter. Note that the infections shown in figures 4 and 5 extend to the outside of the cork.

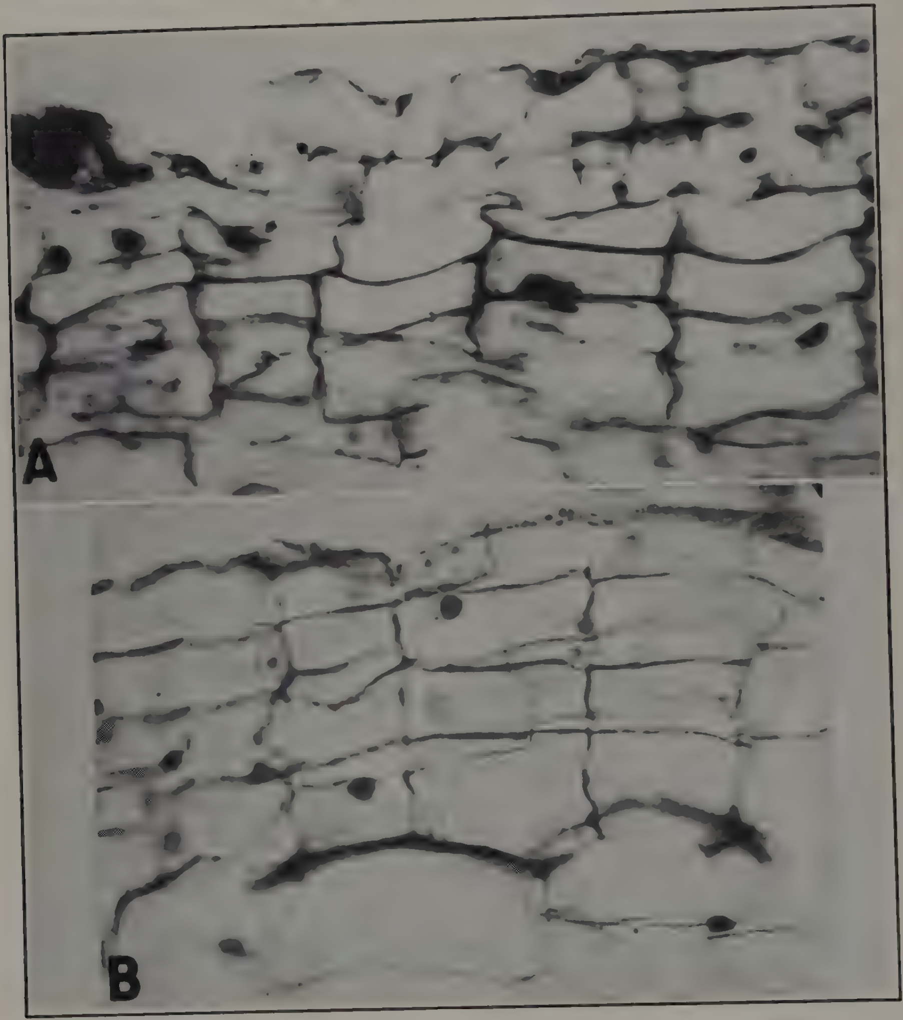

FIG. 4. Sections of cork layers of white (A), and brown (B), skin of young tubers; with cell wall infection, dark intercellular space under cork in No. 9. *Actinomyces* hyphae dark blue, cell walls nonstained, modified Gram's stain. ×400.

Infection of the Stomata and Lenticels. The old and general assumption (shared by the writer, 9, 10) was that stomata or lenticels are the general portals of entry of the parasite. The first browning of the smallest tubers occurs in a zone around these openings, but the infection assumption drawn from it may be fallacious.

Scab lesions are the result of growth in a cambium, a growth much larger under stomata than under the inter-stomatal cork layer. The result is an eruption of brown-walled, irregular, hypertrophied cells—a so-called deep scab lesion.

Stained sections of potato roots showed general infections of the outer layers similar to that described for the tuber.

General Infection of Tuber. Actinomyces filaments were general in the starch parenchyma of all tubers. Figure 6, not drawn at one level, shows all hyphae, large and small, but no cell walls. Cells 1, 3, and 7 are the innermost cork cambium; the others are starch parenchyma. Some of the hyphae are cut off, but a few of the more delicate ones, as in cell No. 4, bend up and then spread out in the plane of the paper but between walls, which, of course, cannot be shown. Even the cells of the rather small and rudimentary bundles showed infected walls. The mycelium in these walls was often very large and coarse, while filaments inside parenchyma cell walls spun out into branches so fine that they could hardly be followed. The question might arise at once: Is this deeply imbedded *Actinomyces* the same species as those found in the walls of the cork and under the scab lesions? The strands seem to be continuous from cork to starch parenchyma.

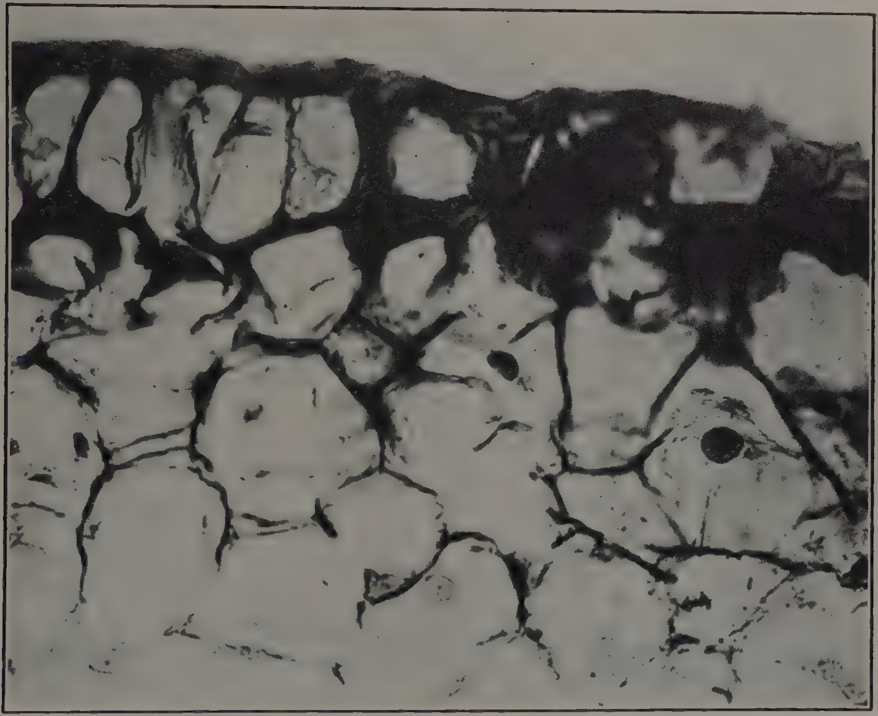

FIG. 5. Section of brown tuber skin with schizogenic spaces between the epidermis; *Actinomyces* hyphae invading the deeper layers of tuber. Modified Gram's stain. × 400.

All tubers, as used above, is to be taken literally. Regardless of scab, the cell walls of all tubers were infested with *Actinomyces* filaments. Clean tubers from various local sources, among them specimens from fields never known to produce scab, and clean tubers obtained in the market from other states were alike infected.

This distribution of hyphae throughout the cell walls of tubers recalls the very significant but neglected contributions of Noël Bernard (3). A fungus characteristic of the potato, *Fusarium solani,* invaded very young roots and tubers to live a semi-symbiotic existence inside the cells. Potatoes grown in sterile soil without a previous Fusarium inoculation grew very few tubers. The *Actinomyces* here described are entirely intercellular and can be seen only by special staining for them.

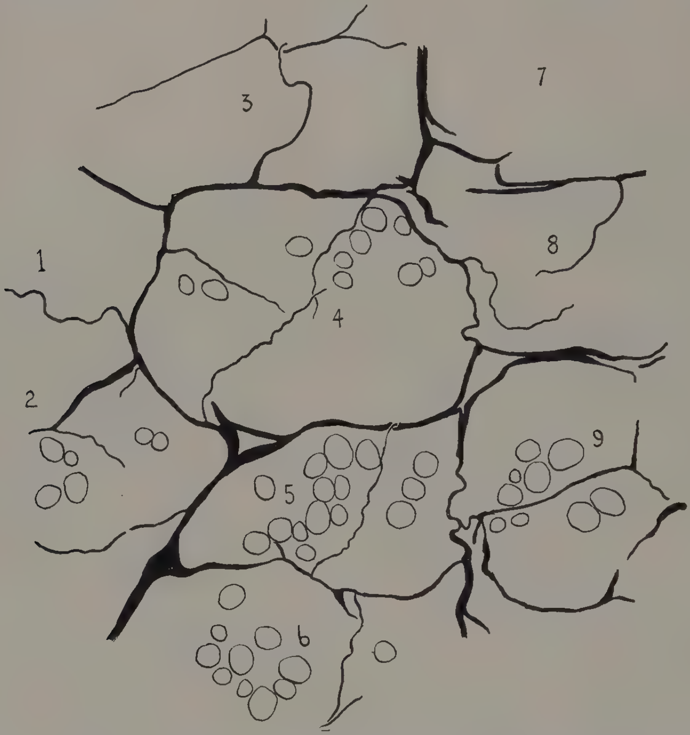

FIG. 6. Starch parenchyma cells, under cork. Modified Gram's stain. ×300.

Beijerinck (1) described actinomycetes in the dead, superficial root cells of various plants. The suspicion would seem to be strong that his so-called "dead cells" were not cells but intercellular schizogenous spaces filled with brown exudate from the adjoining cells. Beijerinck's statements evidently influenced Fellows' (5) explanation of the actinomycetes external and attached to the outer layers of the potato cork, as harmless saprophytes living in some unknown manner.

The *Actinomyces* filaments extend from the cork and cork cambium and can be followed into the body of the tuber. The cork cambium can be stimu-

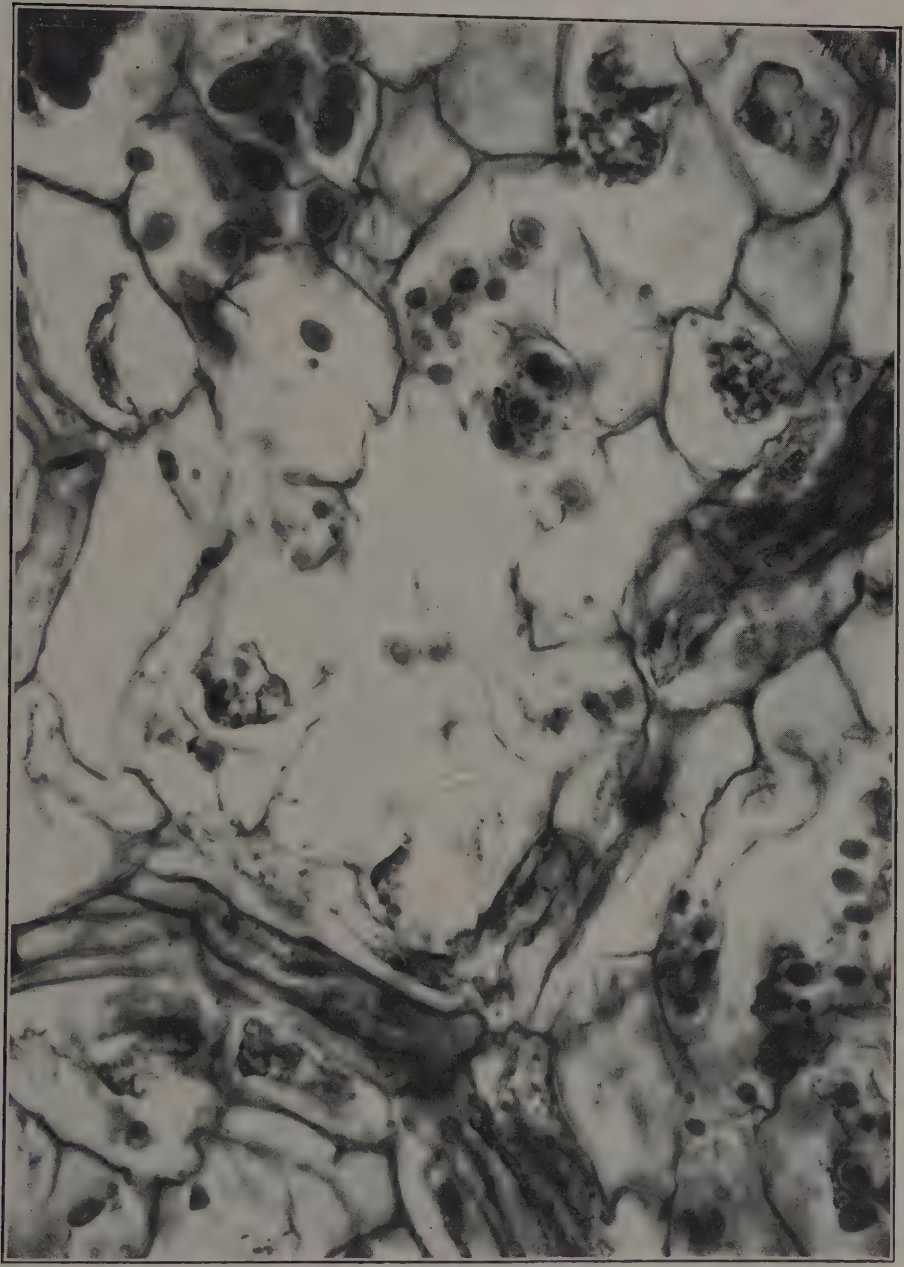

FIG. 7. Starch parenchyma and vascular bundles of tuber. General infection of cell walls. ×750.

lated to abnormal activity; the cambium under the lenticels may be stimulated to abnormal growth. The infection in the stems that later form tubers occurs when they are still too small to be recognized as tubers. Why may not the same growth-promoting substance act on the tissues of the young underground stems? Another pertinent question may be raised as to the extent of *Actinomyces scabies* in these tissues and the means by which it may be distinguished.

THE GROWTH OF ACTINOMYCETES IN CULTURES

The relation of *Actinomyces* to potato-tuber cells led to the possibility that previous attempts at modifications of the ordinary media may have been in a wrong direction, Tempel (14). Growth on nutrient agar is slow and the colonies are small. The agar modifications are too numerous to list. The general function of actinomycetes in soil is the destruction of organic material, but their special method for this destruction is not understood, in spite of much effort. The secret of the proper medium for this group was revealed by their habits on the young potato tubers: They live in the middle lamella of plant cells; they destroy plant remains by dissolving out the middle lamellae. The middle lamella consists of pectin and pectin compounds, so the best medium for soil actinomycetes ought to be pectin.

Since the use of a pectin medium seems to have been limited to that of Wolf (15) from Certo, the following account may be of value. The principal commercial sources of pectin are the gums used in paste, mucilage, and pharmacy, the commonest being gum arabic and gum tragacanth. Apple and orange pectin also are purchasable commercial products.

Gum tragacanth makes a paste in about a 3 per cent solution; the other substances dissolve only into mucilaginous solutions. Gum arabic is nearly a pure pectin; gum tragacanth is largely pectin or pectin-like compounds, but also has a little starch. 1-arabinose sugar, a derivative of pectin, may also be obtained and used.

Various combinations of these pectins were tried, but no combination could be evolved that possessed that valuable property of 1 to 2 per cent agar—solid gel at ordinary temperatures, liquid when hot. The best combination had 4 per cent gum tragacanth as a base with gum arabic, pectin, or 1-arabinose sugar added. The following recipes were found to represent the best proportions:

Formula No. 1

Water (tap)	1,000 cubic centimeters
Gum tragacanth	40 grams
Gum arabic	8 grams
Pectin (apple)	4 grams
$K_2H\ PO_4$	2 grams
$NH_4\ NO_3$	4 grams
$Mg\ SO_4$	0.5 gram
$Ca\ CO_3$	2.5 grams

pH, 6.2

Formula No. 2

Water (tap)	1,000 cubic centimeters
Gum tragacanth	40 grams
Gum arabic	10 grams
l-arabinose	2 grams
$K_2H\ PO_4$	2 grams
$NH_4\ NO_3$	4 grams
$Mg\ SO_4$	0.5 gram
$Ca\ CO_3$	2.5 grams
pH, 6.4	

Colloids, so firm when cool that a filled test tube could be inverted, were soft pastes when hot. Gum tragacanth is not readily soluble, even by continued (hours) boiling. A 2-day soak in tap water with frequent stirring was the best method. Even while hot, the paste was too thick to tube by the usual procedure, or even by pipetting. A stout, heavy-grease pump, such as is used for lubricating automobiles, was the best instrument found. This medium is not quite clear; the slight cloudiness is due to white crystals of unknown composition.

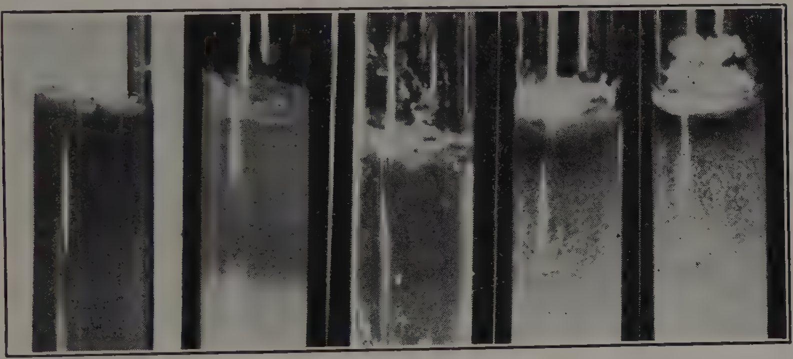

FIG. 8. Chromogenic *Actinomyces* from left to right: On nutrient agar; potato starch agar; pectin—gum arabic—gum tragacanth + peptone medium; same but no peptone; same + l-arabinose sugar.

The actinomycetes used for trials on these media were types isolated on nutrient agar from soil badly infested with scab organisms, but their pathogenicity was not tested. They were, however, pronounced brown-color producers.

Growth on either medium was profuse with numerous conidia, white, with no production of brown color while on nutrient agar; waxy, with few conidia, and brown coloration of medium. After 10 days the upper layer of the medium, under the mycelium, became liquid and clear (Fig. 8).

Inoculated into plates of this medium, colonies were very large (1–2 cm. in diameter), white, flocculent. The medium underneath them was liquefied (Fig. 9, A).

Ten to 20 cc. of medium were pumped into sterile Petri dishes, covering the bottom to a depth of about 0.5 cm. Sterilized by heating in the dry-air oven for about an hour with the cover on the dish and a temperature of about 85° C., the surface of the medium dried, but the water of condensation on the covers was avoided. One cubic centimeter of the 1–1,000,000 dilution of soil was poured over the medium and distributed over the surface as evenly as possible. Actinomycetes colonies could be distinguished by the clearing and liquefaction of the medium around them (Fig. 9, B).

Bits of tuber tissue introduced into these media developed no *Actinomyces*, although they must have been infected. The organism seemed to have established such an intimate relationship to the tuber that it could not be enticed out into similar but artificial surroundings and medium.

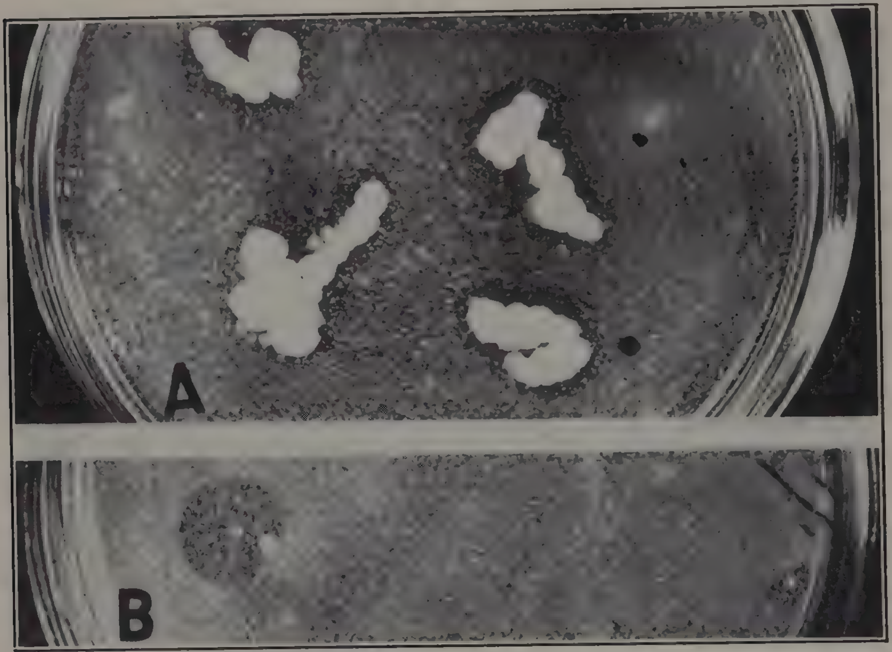

FIG. 9. A. Chromogenic *Actinomyces* liquefying pectin medium. B. Inoculation from scab-infested soil, dilution, 1–1,000,000. *Actinomycetes* colonies with clear liquid halo.

SUMMARY

Infected lenticels showed a ring of brown cells with radiating strands of the Actinomyces in surface view.

In addition to infected lenticels, patches of brown schizogenic spaces were to be observed. Actinomyces strands could be seen inside these spaces and in the intercellular middle lamella; the cells are split apart by the dissolution of the pectin of the middle lamella.

While infection occurs through stomata and lenticels, it seems to occur at any place on the skin where two cells join in very small tubers, 0.5 to 1 cm. in diameter.

In sections of the white skin the hyphae do not produce schizogenic spaces; in brown skin, schizogenic spaces, filled with an exudate, occur under the cork.

The entire tuber is infected by *Actinomyces* hyphae inside of the cell walls. The filaments seem to be continuous from the outer layer of cork to the center of the tuber.

Since the scab organism lives on pectin, a pectin-jelly medium was used. Growth was very profuse, white, with numerous conidia, medium liquefied.

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LITERATURE CITED

1. BEIJERINCK, M. W. Sur la production de quinone par le *Streptothrix chromogena* et la biologie de ce microbe. Arch. Néerland. d. Sc. Exactes et Nat. La Haye (Ser. 3) 3: 337–340. 1900.
2. BRUYN, HELENA L. G. DE. Onderzoekingen over enkele actinomyceten welke aardappelskurft verwekken. Tijdschr. o. Plantenz. 45: 133–156. 1939.
3. BERNARD, N. Études sur la tubérisation. Rev. Gen. de bot. 14: 219–235, 269–279. 1902.
4. DRECHSLER, C. Morphology of the genus *Actinomyces*. Bot. Gaz. 67: 65–83, 147–168. 1919.
5. FELLOWS, H. Relation of growth in the potato tuber to the potato scab disease. Jour. Agr. Res. [U.S.] 32: 757–781. 1926.
6. JONES, F. R. Winter injury of alfalfa. Jour. Agr. Res. [U.S.] 37: 189–211. 1928.
7. JONES, A. P. The histogeny of potato scab. Ann. App. Biol. 18: 313–333. 1931.
8. LIESKE, R. Morphologie und Physiologie der Strahlenpilze (Actinomyceten). Gebrüder Borntraeger (Leipzig). 1921.
9. LUTMAN, B. F. The pathological anatomy of potato scab. Phytopath. 3: 255–264. 1913.
10. ——————. Resistance of potato tubers to scab. Vt. Agr. Exp. Stat. Bull. 215. 1919.
11. ——————, R. H. LIVINGSTON, and ALICE SCHMIDT. Soil *Actinomyces* and potato scab. Vt. Agr. Exp. Stat. Bull. 401. 1936.
12. RAWLINS, T. E. Phytopathological and botanical research methods. J. Wiley & Sons (New York), 156 pp. 1933.
13. SMITH, ERWIN. Bacteria in relation to plant diseases. Carnegie Institution of Washington V. 1, (27). 1905.
14. TEMPEL, ERNA. Untersuchungen über die Variabilität der Actinomyceten. Arch. f. Mikrobiol. 2: 40–71. 1931.
15. WOLF, F. A. Studies on the physiology of some plant pathogenic bacteria. VII. Pectic fermentation in culture media containing pectin. Phytopath. 13: 381–384. 1923.

BROWN ROT ON STONE FRUITS IN WESTERN WASHINGTON

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The brown rot of stone fruits (*Sclerotinia* sp.) does some damage in Western Washington every year, and frequently occurs in epiphytotic form. The disease was especially severe in 1936 when it caused an estimated reduction in yield of 60 per cent in Italian prunes, 40 per cent in sour cherries, and 90 per cent in sweet cherries.

An investigation of brown rot on Italian prunes was undertaken in Clark County in the spring of 1937.[2] The experimental program was enlarged in 1938 to include a study of the disease on Italian prune, apricot, cherry (sweet and sour varieties), and peach.

Two species of *Sclerotinia, S. fructicola* (Wint.) Rehm. and *S. laxa* Aderh. and Ruhl., which have been present in the Pacific Northwest for some time,[3] were found responsible for the disease in Western Washington. Hewitt and Leach[4] found both *S. fructicola* and *S. laxa* on stone fruits in California. The former was isolated most frequently from decayed fruits, whereas the latter more frequently from blighted blossoms and twigs.

It is the purpose of this paper to present a detailed discussion and data pertaining to the host limitations and occurrence of the two species of *Sclerotinia* on stone fruits in Western Washington. A preliminary report of this investigation has been made.[5]

METHODS

Culture Studies

All cultures from isolations were grown on acidulated potato-dextrose agar. *Sclerotinia fructicola* and *S. laxa* were easily identified, as the gross cultural characteristics are distinctly different. Hewitt and Leach[4] pointed out these differences and reviewed the pertinent literature concerning the identification of these two species. Therefore, no further discussion is here included.

Isolations

Whenever convenient, isolations were made in the orchards in order to avoid carrying sporulating specimens long distances to the laboratory. A sedan automobile, with isolation facilities set up in the rear compartment, was driven into the orchards, specimens collected, and isolations made immediately. Otherwise, specimens were placed in large dishes or vials and taken to the laboratory for isolation. From all sporulating specimens freshly developing conidia were removed to agar plates; the specimens were then thoroughly washed in bichloride of mercury solution,[6] dipped into 85 per cent alcohol, and passed through a flame. Isolations were then made from the interior of the specimens. Isolations from apothecia were made by permitting the ascospores to be discharged against the medium.

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[1] The writers express their appreciation to Dr. F. D. Heald and Dr. L. K. Jones for helpful suggestions in the preparation of the manuscript.

[2] Huber, Glenn A., and Karl E. Baur. Progress report of brown rot investigation conducted during 1937 in Clark County, Washington. U. S. Dept. Agr. Bur. Plant Ind. Plant Dis. Rptr. 22: 14–15. 1938.

[3] Barss, H. P. Brown rot and related diseases of stone fruits in Oregon. Oregon Agr. Exp. Stat. Circ. 53: 1923.

[4] Hewitt, Wm. B., and L. D. Leach. Brown rot *Sclerotinias* occurring in California and their distribution on stone fruits. Phytopath. 29: 337–351. 1939.

[5] Huber, Glenn A., and Karl E. Baur. The occurrence of *Sclerotinia fructicola* and *S. laxa* on stone fruits in western Washington. (Abstract) Phytopath. 29: 825. 1939.

Inoculations

Fruits and twigs were inoculated by inserting conidia obtained directly from the host or from pure cultures, or ascospores from mature apothecia, into wounds made by a scalpel. Blossoms were inoculated by (a) atomizing spore suspensions of either conidia or ascospores onto the floral parts; (b) by placement of dry conidia onto the floral parts with a camel-hair brush; or (c) by ascospores discharged directly from apothecia into open blossoms.[7]

SCLEROTINIA SPP. ON APRICOT

The production of apricots in Western Washington is of little commercial importance. Many fruit growers have one to several trees generally planted in cherry, peach, or prune orchards. These apricots are highly susceptible to brown rot and, in some years, most of the year-old twigs are killed in the spring before new twig growth appears (Fig. 1, A). The mycelium within the twigs apparently becomes dormant, new growth appears, and the trees develop good twig growth during the season. These trees are sources of inoculum for infection of cherries, peaches, and Italian prunes.

Studies on the brown rot of apricots were carried on in small plantings within and adjoining peach and Italian-prune orchards in Clark County. Sporodochia were found, from the latter part of December through March, developing in large numbers on twigs infected the previous year (Fig. 1, B, C). When rains and high humidity prevailed during the latter part of March, sporodochia continued to develop into April. Only two mummies, both showing heavy sporodochial development, were found overwintering on trees. No new sporodochial development was observed on twigs and cankers that had produced them the previous year.

New infections took place through spreading blossom buds and leaf buds, and advanced into the year-old twigs, which were girdled and killed. Under highly humid conditions conidial production took place in abundance on blighted blossoms within 2 or 3 days after infection. Sporodochial production was not observed on currently infected twigs and cankers.

Isolations were made in 1938 and 1939 from infected fruit, blighted blossoms, sporodochia (developing on twigs and cankers), and from the interior of blighted twigs. These were collected at random from orchards in widely scattered areas in Clark County. Following are the results of the isolations:

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[6] A small amount of alcohol was added to the bichloride of mercury solution (1–500) to break down the surface tension to aid in penetration of spore masses.

[7] An open flame from a match or an alcohol lamp was passed over the mouth of the apothecium. The drying of the hymenium caused the asci to explode, thus forcibly discharging the ascospores. Large apothecia may be forced to visibly discharge ascospores 4 to 6 or more times in succession.

Sporodochia from 2 mummies (overwintering on trees)—*Sclerotinia laxa.*
Sporodochia from 368 blighted twigs and cankers—*S. laxa.*
Interior of 64 twigs and cankers (current infections)—*S. laxa.*
Over 500 blighted blossoms and blossom-buds—*S. laxa.*

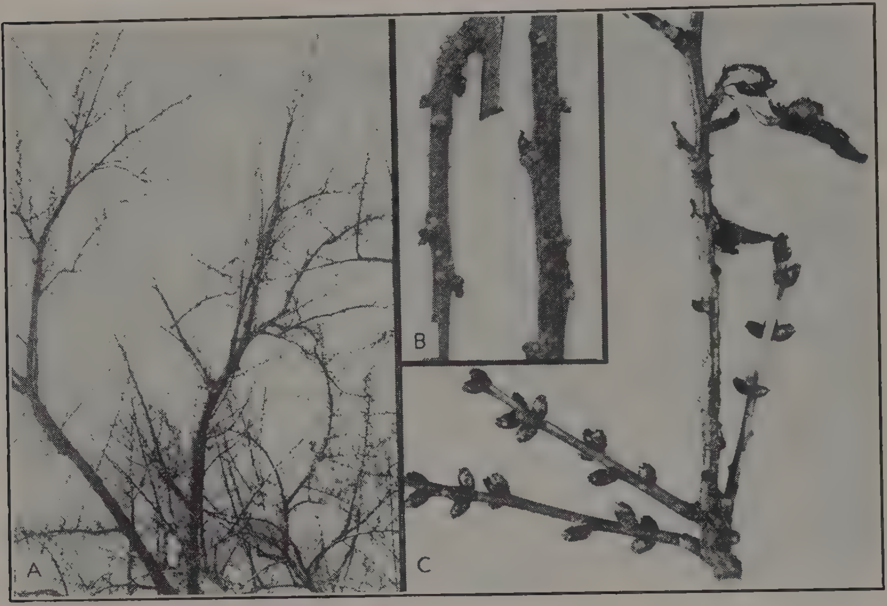

FIG. 1. A. Apricot tree showing twig and blossom blight caused by *Sclerotinia laxa.* B, C. Blighted twigs showing sporodochia of *S. laxa.*

Exterior (conidia) and interior of 72 infected green fruits—*S. laxa.*
Exterior (conidia) and interior of 38 infected ripe fruits—36 *S. laxa* and 2 *S. fructicola.*

Because of the severity of the disease on apricot, very few blossoms either escaped infection or set fruit. Inoculations with *Sclerotinia laxa* caused infection of blossoms, twigs, and fruits. Inoculation with *S. fructicola* caused infection of blossoms and fruits but failed to develop definite cankers in twigs. Blossom infections with *S. fructicola* did not extend into twigs as did those with *S. laxa.*

SCLEROTINIA SPP. ON CHERRY

There are approximately 2,500 acres of cherries (mostly the Montmorency variety) grown in Western Washington. The greater part of this acreage is located in the Puget Sound area. The sweet cherry acreage is small, most of the plantings consisting of only a few trees in or adjoining Montmorency orchards.

The study of brown rot on both sweet and sour varieties was made in orchards located principally in King and Pierce Counties, where most of the commercial crop is grown. Observations also were made and specimens collected in Clark and Whatcom Counties.

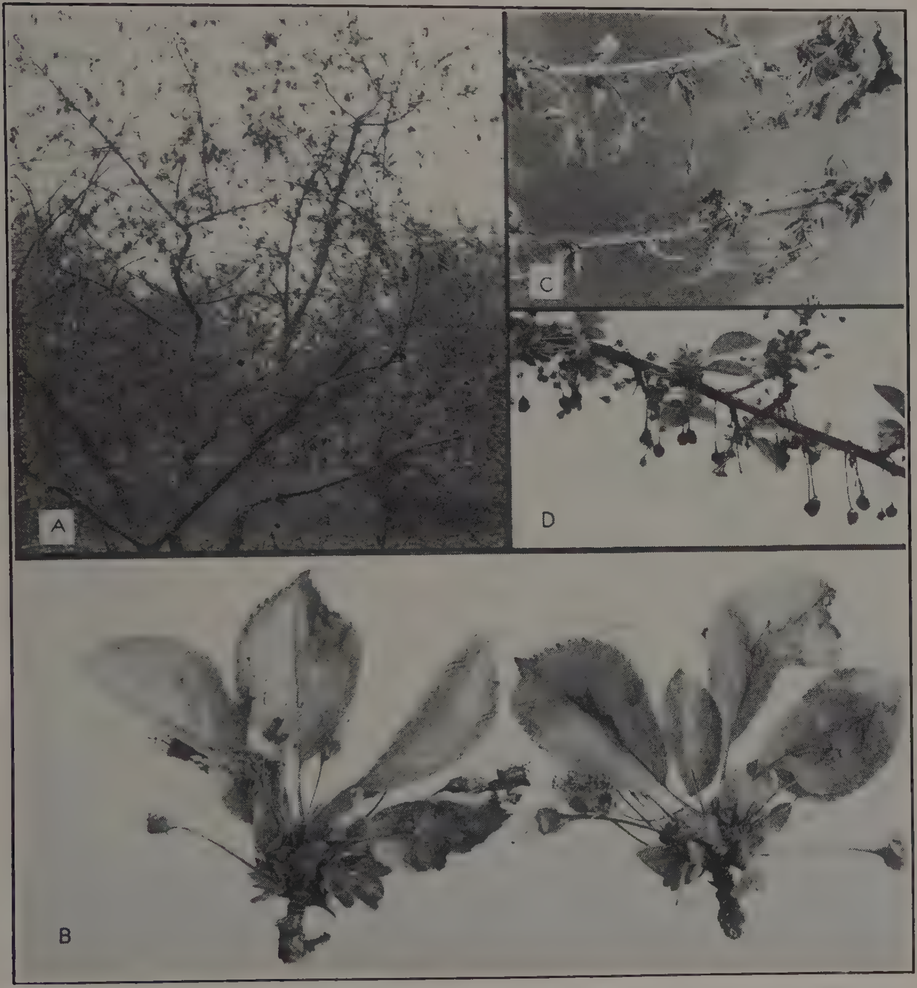

FIG. 2. A. English Morello cherry tree showing twig and blossom blight. Montmorency trees in the background. B. Montmorency spurs showing blossom and leaf blight. C. Early Richmond twigs showing blossom and fruit-spur blight. D. Montmorency twig showing mummies still attached to fruit spurs at blossoming time.

The English Morello and Early Richmond varieties appear to be more susceptible to brown rot (Fig. 2, A, C) than Montmorency. In years of severe epiphytotics, many of the 1- and 2-year-old twigs were girdled and killed. Although the Montmorency is less susceptible, terminal twigs and fruit spurs were often killed. Random examinations of twigs and fruit spurs in 4 different orchards near Kent, King County, in May, 1940, showed killing of 31 per cent of the twig tips, and 31.34 per cent of the fruit spurs. Isolations from infected twigs and fruit spurs yielded *Sclerotinia laxa* only. The greatest damage on Montmorency was to the blossoms (Fig. 2, B) and fruit spurs. The infections advanced down the pedicels and invaded and killed the fruit spurs. Cankers were often found on twigs at the base of fruit spurs. Because of the destruction of fruit spurs and leaf cluster buds by *S. laxa,* shoots with only a few inches of fruit-bearing wood at the tips are found in abundance.

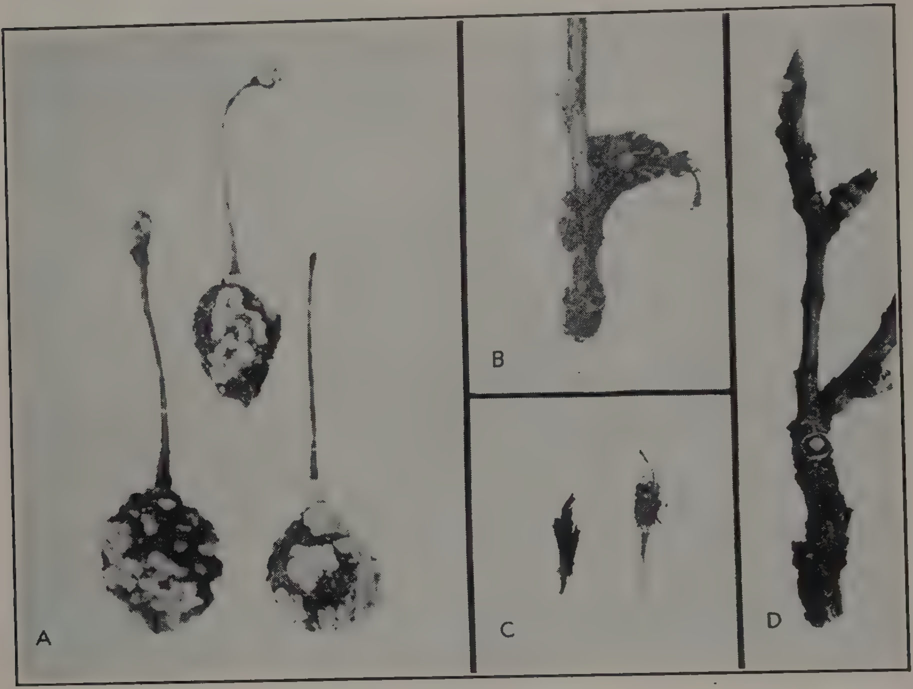

FIG. 3. A. Sporodochia of *Sclerotinia laxa* on mummies. B. Sporodochia of *S. laxa* on fruit spur on 1-year-old twig. C. Sporodochia on blossoms blighted by *S. laxa* the previous year. D. Sporodochia on fruit spur and twig tip blighted by *S. laxa.* (A, B, and C, Montmorency cherry; D, Royal Ann cherry.)

The greatest damage to the sweet varieties was from blossom blight and fruit decay. Spur and twig blight was of little importance.

On both sweet and sour varieties sporodochia were found developing abundantly on cankers, blighted fruit spurs (Fig. 3, B, D), and mummies[8] (Fig. 3, A) hanging on the trees from December through March. Sporodochia were found also on leaf petioles, and pedicels and calyxes of blossom clusters (Fig. 3, C) that had been blighted the previous year and were still remaining on the trees. On February 1, 1939, a count in orchards in the Kent district, King County, showed that fresh conidia were developing on 46 per cent of the mummies hanging on trees. On February 16, 1939, 31

[8] The fruit on trees affected with pink-fruit (Pl. Dis. Rptr. 23: 10–12, 1939) and those affected by the fruit fly and fruit worm usually are left on the trees at harvest (Fig. 2, D). The majority of these become infected with *Sclerotinia* spp.

TABLE 1.—*Results of isolations from sweet and sour cherry varieties in Western Washington, 1938 to 1940, inclusive*

Locality	Variety	Mummies		Fruit spurs and cankers		Blossoms		Fruit	
		S. laxa	*S. fructicola*	*S. laxa*	*S. fructicola*	*S. laxa*	*S. fructicola*	*S. laxa*	*S. fructicola*
Buckley (Pierce Co.)	Montmorency	130	0	45	0	235	18	128	6
	Sweet varieties	......	......	......	......	112	6	120	2
Kent (King Co.)	Montmorency	89	0	89	0	135	170	70	100
	English Morello	......	......	88	0	198	2	......	......
Puyallup Valley (Pierce Co.)	English Morello	18	0	54	0	107	152	......	......
	Montmorency	96	0	21	0	65	95	138	32
	Sweet varieties	48	0	12	0	140	0	108	35
Rosedale (Pierce Co.)	Montmorency	80	0	36	0	36	18	......	......
	Sweet varieties	......	......	......	......	9	72	......	......
Vashon Island (Pierce Co.)	Montmorency	110	0	......	......	......	......	......	......
Lynden (Whatcom Co.)	Montmorency	34	0	20	0	348	12	......	......
Vancouver (Clark Co.)	Montmorency	......	......	......	......	......	......	32	14
	Sweet varieties	......	......	......	......	54	42	34	58

per cent of the mummies in the Rosedale area, Pierce County, showed sporulation. On February 24, 1939, 25.5 per cent of the mummies on Vashon Island, in Puget Sound, were producing conidia.

Isolations were made during the years 1938 to 1940, inclusive, from specimens collected at random throughout orchards in the Puget Sound area and in several orchards in Clark and Whatcom Counties. The results of the isolations are presented in table 1.

Isolations from brown-rot-affected mummies overwintering on trees and from cankers on twigs yielded only *Sclerotinia laxa.* Both *S. fructicola* and *S. laxa* were found responsible for blossom blight and fruit decay. In a few cases, plantings of conidia from blossoms yielded *S. fructicola,* while isolations from the surface-sterilized pedicels of the same blossoms yielded *S. laxa.* Plantings of conidia from the surface of a few fruits yielded *S. fructicola,* while isolations from the interior of the same surface-sterilized fruits yielded *S. laxa.* Since both species were obtained from the same specimens in only a few instances, these were omitted from the table.

Inoculations with *Sclerotinia fructicola* into twigs failed to produce definite cankers. When blossoms were inoculated with *S. laxa* the infections advanced into the fruit spurs and, in approximately 5 per cent of the cases, continued into the twigs, where cankers were formed. *Sclerotinia fructicola* did not advance from blossoms into fruit spurs.

The methods by which *Sclerotinia fructicola* overwinters on cherry have not been determined. Although only limited observations have been made, apothecia have not been found on cherry mummies. Isolations from mummies overwintering on trees, and from twigs and cankers indicate that *S. fructicola* does not overwinter on these parts. However, the high percentage of blossoms infected with *S. fructicola* in the spring indicates that large populations of spores of this species were present in the orchards in some localities at blossoming time.

SCLEROTINIA SPP. ON PEACH

Peaches are not grown extensively in Western Washington. Most of the plantings consist of one to several trees for home use or from 1 to 5 acres for local trade. The smaller plantings usually are included in orchards with other stone fruits.

The investigation on peaches was conducted in Clark County in plantings within or adjoining prune orchards. Abundant sporodochial development was observed from January to mid-April on blighted twigs, cankers (Fig. 4, B), and on mummies (Fig. 4, C and D) hanging on trees. On March 1, 1938, 62 per cent of the mummies hanging on trees showed heavy conidial development, while none was observed on mummies on the ground. Infection took place through blossom-buds and blossoms, and usually advanced into twigs, where cankers were formed (Fig. 4, A). Cankers often girdled the twigs and smaller limbs, causing death to the parts above the cankers. Abundant conidial formation took place on blossoms and decayed fruit. Infection also extended from decayed fruits into twigs (Fig. 4, D) and down twigs (Fig. 4, C). Of the varieties observed Elberta and Crawford appeared to be the most susceptible to blossom and twig infection.

Results of isolations made in 1938 and 1939 from infected mummies, twigs, cankers, blossoms, and fruits were as follows:

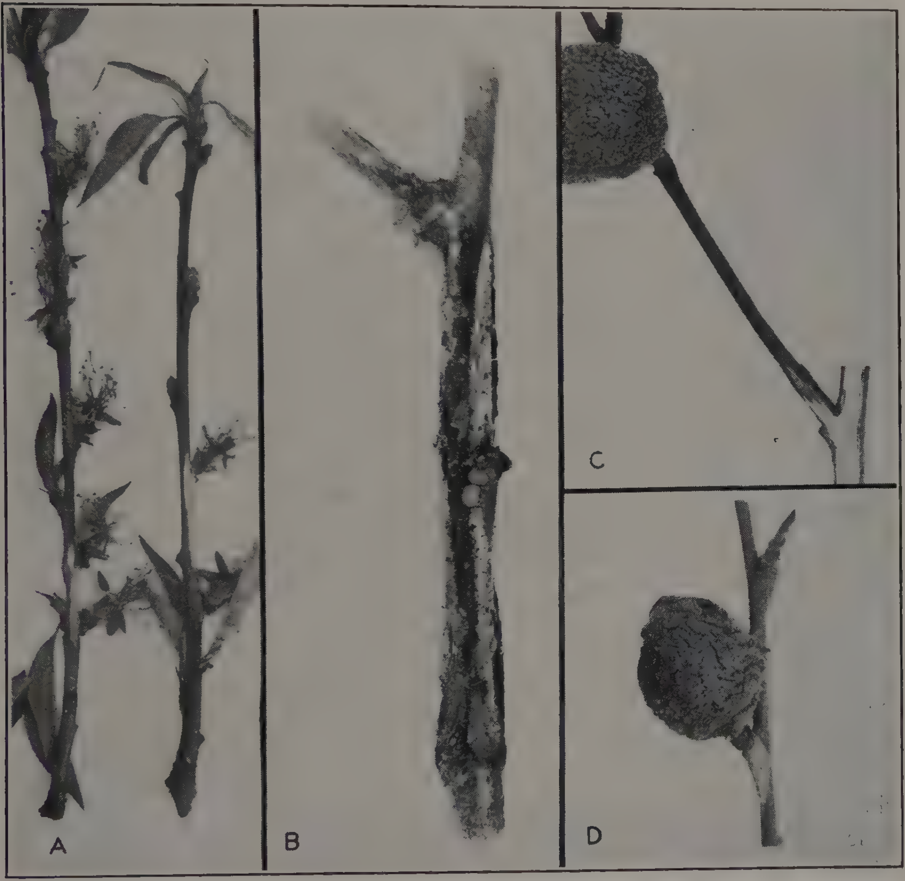

FIG. 4. A. Peach twigs showing cankers at the base of blossoms blighted by *Sclerotinia laxa*. B. Canker on twig caused by *S. laxa* showing sporodochia at base of blighted fruit spur. C. Twig showing distance infection had advanced from mummy caused by *S. laxa*. D. Twig showing canker at base of blighted fruit spur. Attached mummy shows an abundance of sporodochia of *S. laxa*.

From 502 mummies (conidia on the surfaces)—472 *Sclerotinia laxa* and 30 *S. fructicola*.

From 264 twigs and cankers—264 *S. laxa*.

From 276 blossoms—276 *S. laxa*.

From 322 mature fruits—110 *S. laxa* and 212 *S. fructicola*.

Unlike the isolations from cherry mummies, *Sclerotinia fructicola* was isolated from conidia developing on peach mummies that had overwintered on the trees. Isolations from twigs and cankers yielded only *S. laxa*. Inoculations with conidia of *S. fructicola* from prune and peach into 1- and 2-year-old peach twigs failed to produce definite infections, while inoculations with *S. laxa* from peach mummies produced cankers that sporulated abundantly the following spring. Although peach blossoms were blighted when inoculated with conidia of *S. fructicola,* this species was not isolated from naturally infected blossoms. This may be because of the greater susceptibility of blossoms to infection by *S. laxa* or to the absence of a large population of spores of *S. fructicola,* since peaches usually bloom prior to apothecial development of *S. fructicola* in adjoining prune orchards. Apothecia have not been observed developing from peach mummies. As indicated by isolations a greater number of fruits were infected by *S. fructicola* than by *S. laxa.* This may be because of a greater susceptibility of fruit to *S. fructicola* or to the relatively large population of spores of *S. fructicola* that are present during this period of the year. It was not uncommon to obtain *S. laxa* from the interior and *S. fructicola* from the sporulating surface of the same fruit.

SCLEROTINIA SPP. ON ITALIAN PRUNE

There were approximately 7,192 acres of Italian prunes in Western Washington.[9] The industry is localized, with approximately 6,500 acres reported in Clark county. Since 1936 it is estimated that 1,000 to 1,200 acres have been taken out of prune production. The investigation on Italian prunes was carried on in Clark county where brown rot is well established.

MUMMIES

It is well established from previous investigation that mummies affected with *Sclerotinia fructicola,* overwintering on or just beneath the surface of the soil, play an important part in maintaining this species from year to year. However, the part played by mummies overwintering on trees has not been established. Observations of mummies on trees in orchards in 6 widely separated districts during March, 1938, showed heavy sporulation of various fungi. Isolations from conidia developing on over 500 mummies collected from the various orchards showed that *Cladosporium* spp. and *Penicillium* spp. were predominant. *Sclerotinia* spp. were obtained only in orchards in which apricot, peach, or cherry trees were growing. In one orchard where rows of prune and peach trees were interplanted, *S. laxa* was isolated from 11 per cent of the mummies overwintering on the trees (Fig. 5, C).

Mummies, overwintering on trees and showing evidence of *Monilia* sporulation, were collected during the months of March and April of 1938 to 1940, inclusive. One to several plantings of the likeliest looking spore masses were cultured from each mummy. From 465 such mummies, 87 yielded cultures of *S. laxa,* 3 cultures of *S. fructicola,* and 375 cultures of miscellaneous fungi, mostly *Penicillium* and *Cladosporium* species.

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[9] Mimeographed statement of Division of Farm Management and Agricultural Economics, Washington Agricultural Experiment Station, 1937.

Apothecia developing from mummies (Fig. 5, A) and fragments of mummies were found in abundance on the ground under prune trees during April, 1938 to 1940, inclusive. In 1938, the first apothecia were observed April 4, with maximum production around April 15. In 1939, the first apothecia were observed on April 1, none of which was sufficiently mature to discharge ascospores. In 1940, numerous apothecia were observed on March 26; only a few were mature enough for ascospore discharge. Isolations were made from apothecia of different sizes collected in 1938 and 1939 from orchards in 6 localities in Clark County. Apothecia, from 408 mummies, yielded cultures of *S. fructicola* only.

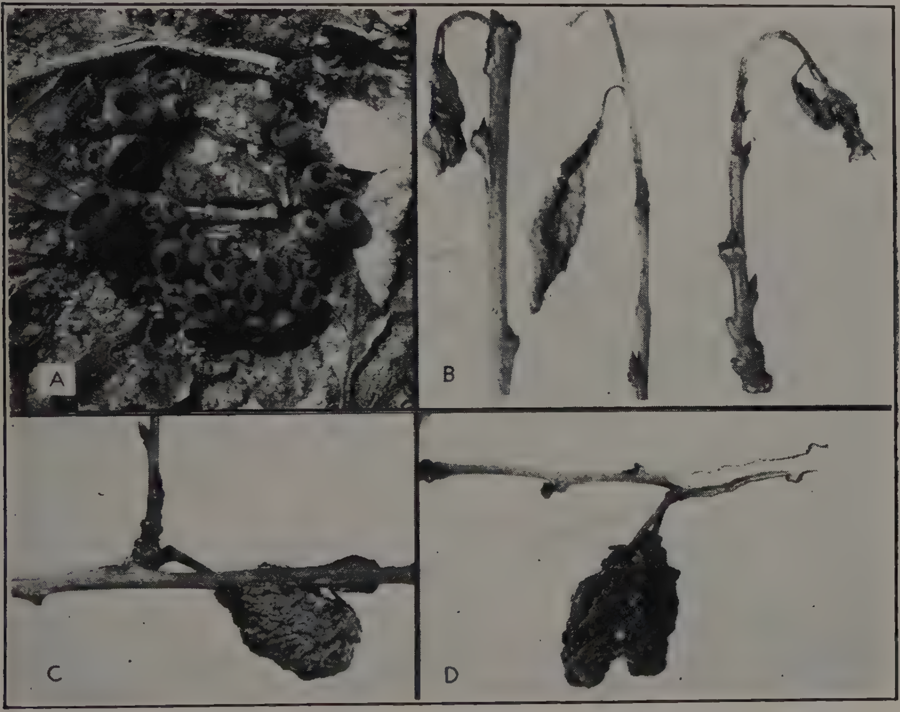

FIG. 5. A. Apothecia developing from mummy overwintering on the ground. B. Tips of Italian-prune twigs blighted by *Sclerotinia laxa.* C. Mummy overwintering on on tree showing sporulation of *S. laxa.* D. Twigs showing dead streak extending downward from point of attachment of mummies. Note lack of *Monilia* sporulation on mummies resulting from infection of *S. fructicola.*

Blossoms

The first blossoms of Italian prune open approximately on the same date that the first apothecia begin to discharge ascospores. By the time the trees have reached full bloom the apothecia have reached their maximum production.

Sclerotinia laxa has not been obtained from prune blossoms, even in prune orchards where peach and cherry trees were planted. It is not definitely understood what part *S. fructicola* plays in causing blossom blight. Conidia are produced in abundance on blossoms that are injured by thrips, *Taeniothrips inconsequens,* and those that normally drop from other causes.[10] Even though conidial formation had been observed on the calyxes of blossoms, fruit continued to develop. Although the calyxes of blossoms showed heavy conidial formation, the organism did not advance from the calyx into and down the healthy pedicels. Hundreds of open and unopen blossoms were inoculated with conidia and ascospores of *S. fructicola,* and conidia of *S. laxa.* The blossoms, especially those inoculated by placing conidia of *S. fructicola* into the open flowers with a camel-hair brush, showed burning on the stamens, styles, petals, sepals, and calyx cups 4 to 7 days after inoculation. There was, however, no evidence of infection advancing down the pedicels. There was no significant difference in the set of fruit on inoculated and noninoculated blossoms. When inoculated blossoms were placed on culture media, those inoculated with *S. fructicola* yielded *S. fructicola,* those inoculated with *S. laxa* yielded only *S. laxa.* However, many of the blossoms inoculated with *S. laxa* yielded mixed cultures of *S. laxa* and *S. fructicola.* When noninoculated, apparently healthy, blossoms, collected from widely scattered orchards, were placed on culture media, from 56.8 to 100 per cent yielded cultures of *S. fructicola.* On June 8, 1938, 1,000 dried blossoms hanging to twigs were collected from various orchards in Fruit Valley and placed on culture media. *Sclerotinia fructicola* developed from 93 per cent of the blossoms. Sections of green leaves, twigs, and bark from larger limbs were placed on culture media with the result that 50 to 85 per cent of the plantings yielded *S. fructicola.* This showed that *S. fructicola* spores were present in great abundance in those orchards in which the isolations were made.

Shedders

The occurrence of shedders, fruits that fail to develop after reaching one-half size or larger, has been reported by the writers (4). The majority of these shedders drop in July and August. From 11.6 to 62 per cent of shedders found on the ground showed *Monilia* sporulation on September 1, 1937. From less than 1 to 3.8 per cent of the shedders on the trees in the same orchards showed *Monilia* sporulation. Conidia from 100 of these sporulating shedders were cultured and all produced *S. fructicola.* There were no apricot, peach, or cherry trees planted in these orchards. A survey of these same orchards just prior to harvest, September 11, showed *Monilia* sporulation on 15 to 46 per cent of the shedders on the trees.

Isolations from discolored pedicels and discolored flesh of shedders not showing *Monilia* sporulation yielded slow-growing, white, cottony, nonsporulating colonies. Identification of this organism, designated as culture No. *S.C. 1,* has not been made.

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[10] Prune trees in Clark County are normally heavily laden with blossoms. Every year a large percentage of the blossoms fail to set fruit and either fall or wither and remain attached to the twigs.

Isolations from the interior of 561 shedders collected at random in 4 orchards in Fruit Valley, and showing *Monilia* sporulation, yielded 285 cultures of *Sclerotinia fructicola* and 276 cultures of the unidentified organism, (Culture No. *S.C. 1*).

Fruit

Infection of immature fruit on trees by *Sclerotinia fructicola* seldom was observed before the fruit turned color and began to soften, even under moisture and temperature conditions favorable for the development of the causal organism. Isolations from immature fruit, showing *Monilia* sporulation, collected from trees in a number of widely scattered orchards during 1938 to 1940, inclusive, yielded only *S. fructicola*. Although *S. laxa* caused decay when inoculated into immature fruit on trees, the development of lesions was slow in comparison with the development of decay caused by *S. fructicola*. Upon maturity the fruit becomes more susceptible to fungous invasion. In Clark County, where much of the fruit is dried, harvest is delayed until the fruit has reached the "soft ripe" stage. The fall rains usually begin a week or two previous, and, from then until harvest, weather conditions are usually favorable for brown-rot development. Infections take place readily through minute cracks in the skin of the fruit that are caused by rain. External conidial formation takes place within 36 hours after infection under warm, highly humid conditions.

The results of isolations made from infected mature fruit collected at random in orchards located in widely scattered districts are presented in table 2.

TABLE 2.—*Results of isolations from infected mature Italian prune fruits in Clark County, 1938 to 1940, inclusive*

Orchard No.	District	Total No. of isolations	Number of *S. fructicola*	Number of *S. laxa*	Number of culture *S.C. 1*[a]
1	Fruit Valley	76	66	10	0
2	Fruit Valley	55	40	5	10
3	Fruit Valley	86	50	0	36
4	Fruit Valley	40	40	0	0
5	Fruit Valley	52	52	0	0
6	Felida	111	111	0	0
7	Felida	131	105	1	25
8	Walnut Grove	27	15	0	12
9	Fischer	54	48	0	6
10	Mill Plain	60	58	0	2
11	Five Firs	32	28	0	4
12	Minnehaha	108	106	2	0

[a] Culture No. *S.C. 1* was isolated from the interior of fruit that showed conidial fructification of *Sclerotinia fructicola* on the surface.

In orchards where apricot, peach, and cherry trees were not included in the plantings only *Sclerotinia fructicola* was isolated, but in orchards that included trees of these other stone fruits *S. laxa* also was obtained.

This strongly indicates that apricots, cherries, and peaches are sources of infection of fruit of Italian prune by *S. laxa.*

Twigs

It was observed that infection extended from brown-rot-infected fruits into the fruit spurs and twigs. Twigs to which decayed fruits (mummies) were attached were collected at random in 5 orchards and examined October 22, 1937, 35 days following harvest. With few exceptions the spurs to which mummies were attached were dead. Discolored streaks usually extended from the dead spurs into and down the twigs. The streaks or discolored areas involved up to one-half or more of the diameter of the twigs, and extended from a trace to 6 or 7 inches below the spurs. In many cases the twigs beyond the place of attachment of the mummies were dead, indicating that the twigs had been girdled at that point. Isolations were made from the discolored streaks of 237 twigs, of which 26 yielded *Sclerotinia fructicola,* 43 *S. laxa,* 35 the unidentified organism (Culture No. *S.C. 1*), and 133 no organism.

On April 5 and 6, 1938, twigs to which mummies of the 1937 crop were attached were collected. Of 557 twigs, 537 showed a definite killing of the spurs with streaks extending down the twigs (Fig. 5, D). Isolations were made from the discolored portions of 136 twigs and neither *Sclerotinia fructicola* nor *S. laxa* was obtained. A number of the twigs yielded the unidentified organism previously isolated from similar twigs.

Sclerotinia fructicola and *S. laxa,* when inoculated into 1- and 2-year-old twigs in October and April of 1938 and 1939, failed to develop definite cankers, although there was evidence that infection had taken place. Surveys in orchards in 1937 to 1940, inclusive, failed to reveal the presence of cankers or twig infections caused by *Sclerotinia* spp. However, during February, 1941, a few scattered blighted twig tips (Fig. 5, B) showing sparse sporodochial development of *S. laxa* were observed in a young Italian prune orchard in which was included a planting of peach trees. Isolations were made from developing sporodochia on 40 prune twigs, all of which yielded cultures of *S. laxa.*

From these studies it is apparent that *Sclerotinia fructicola* is the predominant causal agent of the brown rot of Italian prune, and that *S. laxa* plays a minor role.

SUMMARY

Sclerotinia laxa and *S. fructicola* were found responsible for brown rot of stone fruits in Western Washington.

Isolations were made from mummies (overwintering on trees), apothecia, twigs, cankers, blossoms, and fruits of apricot, cherry (sweet and sour varieties), peach, and Italian prune. The results of the isolations are as follows:

Apricot: S. laxa from twigs, cankers, blossoms, and fruit. *S. fructicola* from fruit only.

Cherry: S. laxa from mummies, fruit spurs, cankers, blossoms, and fruit. *S. fructicola* from blossoms and fruit.

Peach: S. laxa from mummies, twigs, cankers, blossoms, and fruit. *S. fructicola* from mummies and fruit.

Prune: S. laxa from mummies, fruit, and twigs. *S. fructicola* from mummies (.3 of one per cent), apothecia, blossoms, shedders, fruit (green and mature), and twigs to which mummies were attached. Unidentified organism (*S.C. No. 1*) from shedders, mature fruit, and twigs to which mummies were attached.

Inoculations into blossoms, twigs, and fruit of apricot, cherry, peach, and Italian prune were made with *S. laxa* and *S. fructicola.* Both species caused blossom blight on apricot, cherry, and peach; and, although causing infection of various floral parts of Italian prune, no infection was observed to develop into and down pedicels. *Sclerotinia laxa* caused definite twig cankers on apricot, cherry, and peach, but not on Italian prune, while inoculations with *S. fructicola* failed to produce definite cankers. Both *S. laxa* and *S. fructicola* caused decay in fruit of apricot, cherry, peach, and Italian prune.

Sporodochia of *S. laxa* were found on cankers, blighted twigs and fruit spurs, and mummies overwintering on trees of apricot, cherry, and peach, and on mummies and blighted twigs of Italian prune.

Apothecia of *S. fructicola* were found in abundance developing from Italian prune mummies. Apothecia were not observed on cherry or peach mummies.

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BLISTER RUST STUDIES OF THREE PATCHES OF RED CURRANTS IN NEW YORK

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In New York, for the past few summers, the writer has been making a study of the question of the menace of cultivated red currants[1] to *Pinus strobus* because of the blister-rust fungus *Cronartium ribicola* Fischer, and consequently of the necessity or desirability of eradicating these currants from gardens. One line of attack upon the problem has been the relative volumes of sporidia produced by European black currants (dangerous to white pine at a mile and a quarter to a mile and a half), wild gooseberries (eradicated only up to 900 feet or less, under ordinary conditions), and garden red currants (also supposedly dangerous up to within 900 feet of pine.) It has, of course, been long realized that red currants are nowhere nearly so susceptible to attack by this fungus as are black currants, or even wild gooseberries (5, pp. 21–24). It has been known also that there are factors inimical to the production of telia and pine-infecting sporidia, which would be expected, even from the small relative amount of infected leaf-area of red currants. Some of these factors are (a) the high degree of resistance of mature leaves of these plants (1, 2); (b) the tendency to produce a single set of leaves a season (1, 2); (c) early defoliation of the bushes (4); (d) necrosis of blister-rust spots (5, p. 24 and pl. 5); (e) decreased number of uredia and telia (5, p. 24); and (f) lowered viability of teliospores and sporidia (5, p. 24). As the recent study progressed, these factors assumed greater importance, and it became apparent that more information on the general subject would be of value, especially since data of a quantitative nature of the desired sort were lacking.

The present paper makes no attempt to discuss the subject exhaustively, but presents data obtained from a detailed study of 3 patches of red currants in the Adirondacks. A more complete investigation will follow.

THE LOON LAKE CURRANTS, WARREN COUNTY

The data on these currants illustrate two points, *i.e.*, the susceptibility of only a small number of the bushes in a currant patch; the early dropping of infected leaves and the early defoliation of red currants, especially of infected bushes.

This patch consisted of 6 bushes, of about 2,000 to 4,500 leaves, in a well-cultivated garden. Only one bush ever showed any appreciable infection; the other bushes remained infection-free from season to season or produced a few—25 to 75—infection spots. This situation is not an unusual one; in fact, it has been the rule. In both the Adirondack region and in the western counties, never more than 2 out of a row of 6 or 12 bushes in a garden have been found more than very slightly infected (a few spots per bush), and often not more than 1 out of 12. The explanation of this situation is of no interest here, but the application of the fact is of far-reaching importance. In the past, a patch of red currants has been considered "infected" if a single blister-rust spot could be found upon a few of the bushes, and "infected bushes" have been considered as a source of danger to white pine. The important point in the danger of *Ribes* to white pine is the volume of sporidia produced by the former. In addition to the usual low susceptibility of any red currant bushes, the susceptibility at all of only 1 or 2 bushes in a garden row is a very important factor in further reducing the potential volume of infective sporidia.

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[1] In this paper the term "red currants" should be interpreted as referring to the cultivated garden currants of mixed parentage. These horticultural plants have originated from 3 species: *Ribes sativum* (Rchb.) Syme (= *R. vulgare* Lam.), *R. rubrum* L. and *R. petraeum* Wulf. Hybrid varieties of our *sativum* are generally slightly susceptible to the white pine blister rust, whereas those originating from *R. rubrum* and *R. petraeum* are highly resistant to, or perhaps immune from, this disease. Similarly, "black currants" refers to the highly susceptible cultivated black currant of Europe, *R. nigrum* L. Our native swamp red currant (*R. triste* Pall.) and the American black currant (*R. americanum* Mill.) are not considered in these investigations.

Concerning defoliation of the various species, Metcalf (4) stated that red currants produce telia over a shorter period than do other species of *Ribes*. York *et al.* (7) reported that, in 1918, in North Conway, New Hampshire, both black and red currants, "heavily infected," had become defoliated by the disease by the middle of August. Spaulding (6), however, reported the collection of vigorous green leaves as late as September 29 on Block Island, Rhode Island, and in New York. Somewhat more extended data are available on the black currants because of the use by the writer of black-currant material for inoculation purposes over several years, as well as because of a special interest in these plants. One patch in the Adirondacks observed from 1923 to 1927 was not defoliated until the middle of September in any year. Two other lots in the same region were observed from 1930 to 1938. In 1930, these 2 lots retained the bulk of their leaves and maintained their high degree of infection until September 1; then the leaves began to fall and to become inferior for inoculation purposes. In 1931, the bushes were normally vigorous until September 6. In 1932, the corresponding date was August 20, in 1936 it was September 1, and in 1937 and 1938 it fell on September 6. The latter date was the last on which they were examined in any year.

The situation is about the same with wild gooseberries as with black currants. Very rarely, because of drought, leaf-eating larvae, and leaf-spot diseases, the bushes may be defoliated by mid-August. As a rule, however, defoliation does not occur until late September. Not infrequently, even in northern New York, the bushes may retain almost all their leaves until the ground freezes in October. Spaulding (6) mentions leaves of *Ribes cynosbati* which remained on the bushes until December.

On the other hand, in the Adirondacks, the red currants produce telia in quantity over a shorter season; in fact, the higher the percentage of infection the earlier the leaves drop, the more heavily infected leaves falling first.[2] Figure 1 presents the data on 2 of the bushes side by side in the Loon Lake garden in 1938, one heavily infected for red currants, and the other not infected at all. This is not an isolated case, but one of many similar situations.

Important points to note in figure 1 are the following: (a) The sudden decrease in the number of leaves on the infected bush after August 1 as compared with the noninfected bush. (b) The decrease in leaf area and in the amount of infected leaf area on the infected bush between July 25 and August 3, prior to the pronounced decrease in the total number of leaves on the bush. This condition was the result of the loss of all the most heavily infected leaves in those 9 days, many of which were of the larger sizes. (c) The pronounced decrease in the amount of infection from August 3 to 19 from 4.6 per cent to 1/40 of 1 per cent, covering only 2.04 sq. in. of leaf surface, as compared with only a 30 per cent reduction of the number of leaves on the bush in the same period.

Another point of interest will explain the one prominent irregularity in the curves—the hump in the curve for the total leaf area of the infected bush between August 15 and September 1. This hump means that there was an unexpected irregularity of decline in the total leaf area for that period, while there was not this discrepancy in the number of leaves in that period. The explanation is that as a large number of old, chiefly infected, leaves fell after mid-August, their places were taken by new leaves that made the total number on August 19 appear about as expected from the curve. The total area, however, was larger because the rapidly expanding new leaves attained a larger size than did their predecessors.

In this connection, it should be noted that here is evidence that the red currants, at least occasionally, do form a second set of leaves during a season, especially on bushes that are infected to the maximum degree. On noninfected or more lightly infected bushes, this is not the case (2).

As an incidental fact from the data on this lot of bushes, it is observed that the percentage of infection of the infected bush was 6.6 per cent, on an area basis. This was the highest infection for red currants noted by the writer in New York State up to the time of the study of the plot.

THE CROWN POINT CENTER CURRANTS, ESSEX COUNTY

In the summer of 1940, Blister-rust Foreman Charles Cleland showed the writer some red currants on the American Steel and Wire Company property, 7 miles west or northwest of Crown Point Center. This row of currants consists of 10 uncared-for bushes along a wall on an abandoned farm. These bushes are in a clearing that runs approximately east and west, about 180 ft. wide, extending 300 ft. west of the bushes and 550 ft. east. The clearing slopes up-hill to hardwoods on the north, up-hill to an old field over the hill to the west, down-hill to an alder brook on the south and is level to hardwoods on the east.

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[2] On the red currant bushes herein reported, infection of leaves by other fungi, such as *Gloeosporium*, was so light as to be of no consequence from the standpoint of defoliation.

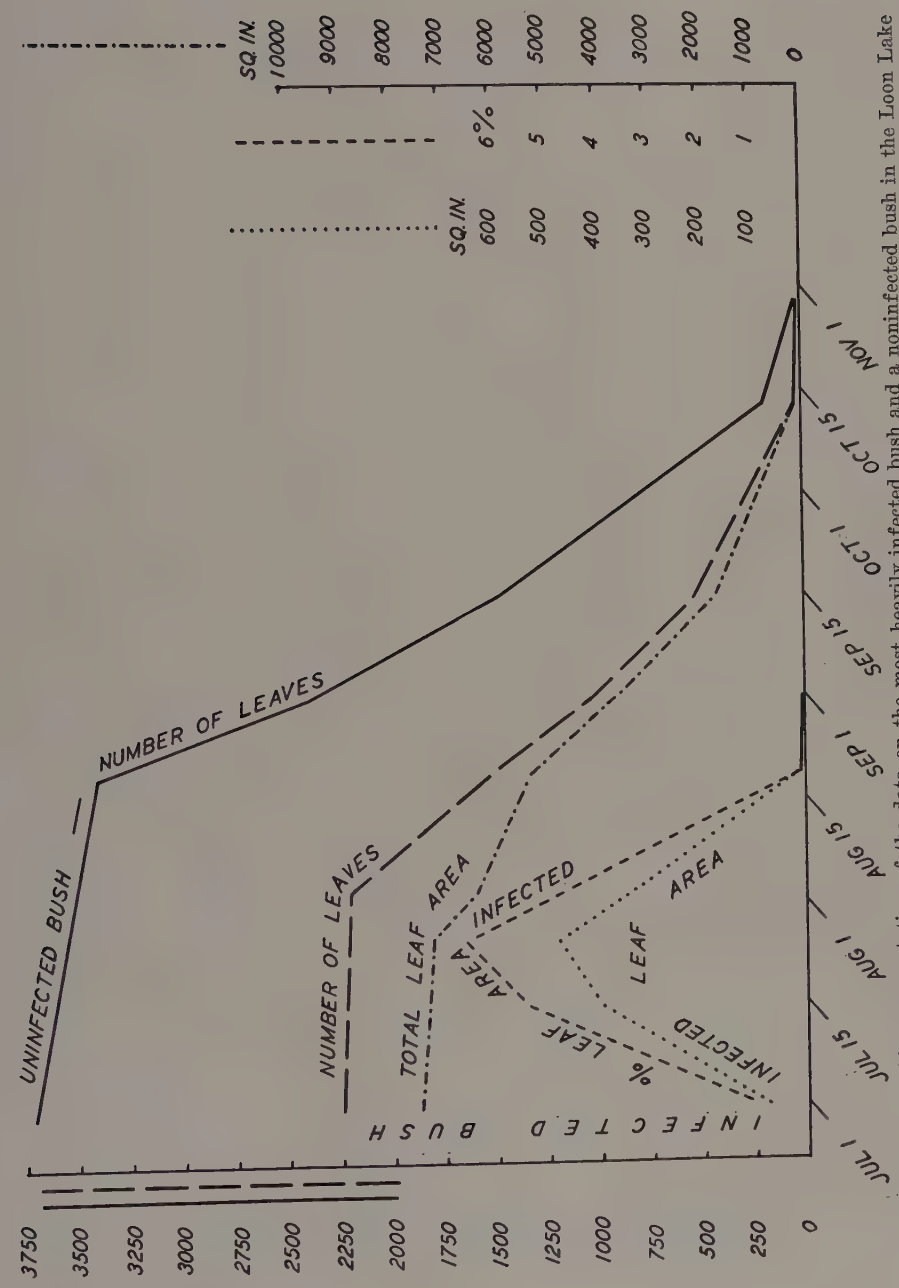

FIG. 1. Graphic representation of the data on the most heavily infected bush and a noninfected bush in the Loon Lake currant patch. Warren County.

When first inspected—early July—it was obvious that there was much of interest in this patch of currants. Some of the bushes seemed very heavily infected, for red currants, the leaves were apparently falling rapidly,[3] and a great deal of the infected area appeared to have died without the production of telia. Accordingly, rather detailed studies were made on July 17 and 30.

In the first instance it was found that 4 of the 10 bushes were 5 to 6.7 per cent infected, 4 were 2 to 3.5 per cent infected and the other 2, .7 to 1.5 per cent infected. The 10 bushes bore 286 to 5,415 leaves, with a total of 15,746, and a total area of 39,731 sq. in. Of this total area 1,236.79 sq. in. were covered with blister-rust spots, or 3.1 per cent. This figure is higher than any found in previous studies (2.2 per cent), but it must be kept in mind that these bushes have received no care for years. In the writer's experience, uncared-for red-currant bushes, as well as red currants that escaped from gardens, are more heavily infected than well-cultivated bushes. Bush no. 3 was the most heavily infected. On precisely 1,000 leaves with a total area of 2,340 sq. in., 18,557 infection spots covered 156 sq. in. for less than 6.7 per cent infection, the heaviest infection ever found by the writer on a red-currant bush, but by less than .1 per cent.

On July 30, it was observed that over 35 per cent of the leaves in the patch had fallen and that the infection of those remaining was less than 2 per cent, even with new infection spots formed in the intervening period. As before indicated, the more heavily infected bushes lost the largest numbers of leaves. For example, bush no. 1, originally found to be .7 per cent infected, lost 3.4 per cent of its leaves; bush no. 10, 1.5 per cent infected, lost 18 per cent; bush no. 9, 2 per cent infected, lost 47 per cent; and the other bushes, 3.5 to 5.5 per cent infected, lost 47 to 77 per cent of their leaves (bush no. 3 had been pulled up before July 30 for more detailed investigation).

When first considered, the most noticeable feature of these bushes was the apparently tremendous proportion of the infected area that was necrotic or, even though alive, was producing few uredia and telia. The extent of necrotic area on all the bushes varied from 68 to 93 per cent, with a figure of 78 per cent for the entire row. Careful studies were made of bush no. 3, with 83 per cent of its infected area necrotic. Of the 156 sq. in. infected, 131 sq. in. were dead and bore no telia, and 25 sq. in. alive, with about 10 sq. in. bearing telia and 15 bearing uredia. On the 10 sq. in. with telia, the latter appeared to be dead. Whereas, in other studies, it has been figured that red currant under optimum conditions produces 2400 telia per sq. in. of infected area, on this bush it was found that there were only 6488 on the entire bush, or 41 per sq. in. of infected area. In other words, this bush, with a low infection percentage, as compared with black currant or wild gooseberry, had its potential production of sporidia further reduced by about 64 per cent because of the premature dying of the infection spots before telia and, sometimes, even uredia, could be produced. Fifty-six sq. in., or 36 per cent of the infected area, had formed neither uredia nor telia before the spots died, and 10 sq. in., or 6.5 per cent, had only 2 to 5 uredia per infected spot.

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[3] See footnote 2.

This 78 per cent of infected area of all bushes in the row, found to be necrotic on July 17, appears tremendous, but, on July 30, it had increased. On the 9 remaining bushes (without bush no. 3, which had been removed), 93 to 100 per cent of the infected area was dead, or 96.5 per cent for the entire remaining row.

Another point of interest and of importance in this vital matter of the reduction of potential sporidia production by red currant is the shortness of the telia found on these Crown Point bushes. Careful counts, especially on bush no. 3, showed that 95 per cent of the telia present were short ones—22 to 50 per cent of the length of normal ones on these bushes and others in other localities. Telia, considered as normal, were 800–1360 μ long, whereas these short ones were 300–400 μ long. Furthermore, there is no knowing whether these short telia were viable or not, since germination studies of the teliospores could not be made at that time. All that can be said is that a goodly proportion of the telia appeared to be dead, if color and the much shriveled form are criteria. At least, it was determined that on most of these short telia, the teliospores had not germinated prior to the time of examination.

There is another point of interest in the data from this lot of currant bushes. In the earlier observations it was found that the older dead spots on the leaves averaged .0112 sq. in., while the fresh, live ones on the leaves at the same time, bearing uredia and perhaps telia, averaged .00347 and .0038 in two sets of measurements. Many of these later-formed spots were so small that they bore only a single uredium. The interpretation of these facts would appear to be that in the case of the spots formed earlier, the fungus encountered less resistance and formed larger spots, while, on the other hand, as the leaves matured they became more resistant, as has been quite generally accepted for a long time (2).

THE JOHNSBURG RED CURRANTS, WARREN COUNTY

Another patch of red currant, 6 in number, was located on an abandoned farm in Johnsburg in July, 1940. These bushes were studied in much the same manner as the lot just discussed, but not in so much detail.

Three of the bushes were 3 to 5 per cent infected and 3 were .05 to .1 per cent; the infection for the entire row was 2.6 per cent. Again, as in the Crown Point Center currants, 3 of the 6 abandoned bushes were more heavily infected than is the case with bushes in well-kept gardens.

It was observed that the leaves were dropping at about the same rate and under the same conditions as in the Crown Point patch. The necrotic area ran from 74 to 100 per cent, and was 75.6 per cent of the total infected area for the entire row. The situation with regard to the shortness of the telia was the same as that noted for Crown Point Center—about 95 per cent of them being equally short.

DISCUSSION

Since this paper consists of a presentation of data on 3 lots of red-currant bushes in New York, it may be well to discuss the general applicability of these facts to the entire red-currant situation.

It already has been stated that the failure of 4 or 5 out of 6, or 10 or 11 out of 12, bushes in a garden patch to become infected is nothing unusual, but quite the rule as found since 1923, both in the Adirondacks and in the western counties. In this connection, attention should be called to the words "in a garden"—referring to bushes that are more or less cared-for, on cultivated soil. The statement that never more than 1 or 2 red-currant bushes in a row or patch are infected heavily for red currant does not apply to uncared-for, uncultivated bushes on abandoned farms, etc., for it has been found that such red currants are as a group always more heavily infected than cultivated bushes. The same is true for *Ribes sativum* plants growing wild (commonly called "escaped bushes" or "escapes").

The low degrees of infection of red currants exemplified here are unusual only in that they are rather high for the writer's experience in the Adirondacks, and very high for the western counties.

The early dropping of infected leaves and advanced defoliation of red currant bushes are general phenomena; the behavior of the plots here reported is typical.

With regard to the necrosis of blister-rust spots on red currant, the situation may be different. This phenomenon was not studied by the writer before 1940, although he has had a general idea from casual observations that from 25 to 50 per cent of the spots on these plants ordinarily become necrotic before the production of telia and sporidia. The two lots of currants reported here as having a tremendously high mortality of the blister-rust spots both consisted of uncared-for bushes on abandoned farms, and were studied during the same season. There is no way of knowing whether this extreme degree of necrosis is because of lack of care of the bushes—their somewhat wild mode of life over the last few or possibly many years—or whether such a high degree of necrosis is attributable to seasonal or climatic phenomena. Kimmey states (3) that necrosis of spots is most acute in dry seasons. It so happens that in the Adirondacks in late June and during July, 1940, while very cool at first and then very hot and humid, there was much more rain than for several years. For example, in Warrensburg and vicinity, there was some rain on 16 days of the 31 in July. There was a very light shower on one day, there was one all-day rain, and on the other 14 days, the rain varied from a very heavy shower to several showers or steady rain for 2 to 12 hours. In the last 2 weeks of July, the growth of fleshy fungi in the woods was much more prolific than in any season since the very wet ones of 1926 and 1928.

In any event, the facts on necrosis of spots are given only for what they are worth; before general conclusions are drawn, these facts should be supported by similar results obtained elsewhere and over a period of time. On the other hand, most blister-rust workers have noted the necrosis of infection spots for many years, and some have commented upon them. It is very important that the dying of these infection spots before the formation of telia, or before the telia may germinate, or even before uredia can be formed, should be given serious consideration in making generalizations about the danger of red currants to white pine. It is certain that, at least upon occasion, through necrosis of the infection spots, the blister-rust fungus on red currants may be reduced to complete impotence from the point of view of its ability to produce sporidia, and hence, to infect pine.

In general, the same may be said of the data upon short telia on the last two lots of currant discussed, as was said of the factor discussed just above. It is not known how general this situation is on red currants; but, again, this factor ought to be given due weight in any study of the epiphytology of red currant and in conclusions concerning their relation to the destruction of white pine.

In any event, all the factors here discussed are very important in reducing the total volume of sporidia produced on the relatively resistant red currants and it appears to be more than likely that in the vast majority of cases, sporidia production by red currants may be so reduced as to make these bushes of little moment in the infection of white pine. It should be kept in mind also that there are other operating limiting factors that are not discussed in this paper.

SUMMARY

From the point of view of the white-pine blister rust, the most important aspect of any species of *Ribes* is its susceptibility to the blister-rust fungus and the consequent potential volume of sporidial inoculum. As compared with other species, the relatively resistant red currants are more affected by various factors that limit the production of this inoculum. The results of studies of 3 patches of currants are used to illustrate certain of these factors.

It is shown that only 1 or 2 bushes in a row of 6 or 12 are more than very slightly, if at all, infected. It is shown that infected red-currant leaves are dropped very early in the summer, that red-currant bushes are defoliated much earlier than black currants or wild gooseberries, and the earlier, the more infected they are. In other words, the blister-rust season for red currants is customarily a short one.

In 2 lots of neglected currants growing on abandoned farms, 75 to 96 per cent of the infection spots were necrotic before the end of July, 1940, before telia and, sometimes, even uredia, could be produced. On 1 bush, only 6468 telia were observed, or at the rate of 41 per sq. in., instead of 2400 per sq. in. under optimum conditions. Furthermore, on these bushes 95 per cent of the telia were only 22 to 50 per cent as long as those considered normal for red currant, and most of these apparently were dead.

On the bushes of these same 2 patches, the earlier-formed, dead infection spots were .0112 sq. in. in area, while the fresh, live spots averaged about .0036 sq. in., with some of them so small that they bore only a single uredium.

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LITERATURE CITED

1. HAHN, GLENN GARDNER. The inoculation of Pacific northwestern *Ribes* with *Cronartium ribicola* and *C. occidentale*. Jour. Agr. Res. [U.S.] 37: 663–683. 1928.
2. ————. A physiological method of distinguishing *Cronartium ribicola* and *C. occidentale* in the uredinial stage. Jour. Agr. Res. [U.S.] 40: 105–120. 1930.
3. KIMMEY, J. W. Susceptibility of *Ribes* to *Cronartium ribicola* in the West. Jour. For. 36: 312–320. 1938.
4. METCALF, HAVEN. The red currant question. Report of the Proceedings and Recommendations of the 9th Annual Blister Rust Conference (mimeographed), Boston, Massachusetts (1924): 107–112. 1924.
5. SPAULDING, PERLEY. Investigations of the white pine blister rust. U. S. Dept. Agr. Bull. 957. 1922.
6. ————. Viability of telia of *Cronartium ribicola* in early winter. Phytopath. 12: 221–224. 1922.
7. YORK, HARLAN H., WALTER H. SNELL, MINNIE W. TAYLOR, and ANNIE RATHBUN-GRAVATT. Experiments with the stages of *Cronartium ribicola* which occur on *Ribes*. Unpublished manuscript, Office of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. 1927.

SPORULATION INJURY ASSOCIATED WITH DOWNY-MILDEW INFECTIONS

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(Accepted for publication December 2, 1940)

Sporulation of downy mildews involves a growth and maturation of the sporangiophores and sporangia during a few hours at night. This process results in injury to the host, as has been recorded by McKay[2] for the downy mildew of onions. Quantitative measurements of this injury and attempts to determine its cause are here reported.

MATERIALS AND METHODS

The downy mildew of onion (*Peronospora destructor* (Berk) Caspary), the downy mildew of hop (*Pseudoperonospora humuli* (Miy. and Tak.) Wilson), and that of spinach (*Peronospora effusa* (Grev.) Ces.) were used in this study. For quantitative observations artificially inoculated leaves or pots of plants were paired or suitably grouped before the treatment involved in this study. The downy mildew of hop was cultured on detached leaves in the laboratory, and opposite leaves were used as paired samples for contrasting treatments. The other downy mildews were cultured on potted plants in the greenhouse.

MAGNITUDE OF SPORULATION INJURY

Onion leaves on which downy mildew sporulation has occurred die sooner than similarly infected, but nonsporulating, leaves. Differences in green weight of infected tissues that had sporulated and in that of comparable tissues that had not sporulated were used as a quantitative measure of sporulation injury. To induce sporulation infected plants were incubated overnight in dark moist chambers at about 15° C., under which conditions the fungi studied usually sporulated luxuriantly. The control plants were held on the dry greenhouse bench and did not sporulate. In some tests the control plants were sprayed with 2 per cent lime-sulphur or 2 per cent rosin-lime-sulphur, and incubated in the moist chamber with the nonsprayed plants. The lime-sulphur was effective in preventing sporulation, but caused host injury in some tests. Its use was discontinued. The vapors of benzene were applied in some tests to inhibit sporulation, but the effectiveness of the vapors from dilute lime-sulphur in inhibiting sporulation of several downy mildews was not known at the time most of these tests were conducted. Before and after incubation for sporulation the sporulating and nonsporulating plants were maintained in the same environment. After an arbitrary period following sporulation the green weight of the test leaves or plants was determined. In the tests prior to April 15, 1938, paired pots of inoculated seedling onions were used as the test material, and all plants in each pot were harvested by cutting them at the ground line. In 8 such tests with 1 to 15 pairs of pots in each test the green weight per pot of plants incubated for sporulation averaged from – 2 to 24 per cent, with an average of 13 per cent less than plants that had not been incubated to induce sporulation. In such tests, however, there usually were some noninfected plants; and, as the growth of these plants was greater than that of the infected plants, this method gave small differences with large errors.

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[1] The writer acknowledges the assistance of J. F. L. Childs and H. Schneider in making some of the measurements reported here.

[2] McKay, R. Observations on onion mildew caused by the fungus *Peronospora schleideniana* W.G. Sm. Jour. Roy. Hort. Soc. **54**: 272–285. 1939.

All tests subsequent to April 15, 1938, are summarized in table 1. In the test of April 15 the plants were incubated on the day before weighing and the few nonsporulating plants were discarded. The green weight of the infected plants is given as yield per pot or yield per plant. The yield per living plant indicates a smaller difference, because of sporulation, than the yield per pot because some of the smaller plants on which sporulation had been induced had died. In the test of May 21 locally infected onion leaves on plants grown from bulbs were paired before sporulation and each leaf was marked 10 cm. from the tip. Five days later, at which time the sporulating leaves were mostly collapsed while the nonsporulating leaves were turgid, the leaves were cut off at the mark and weighed. The green weight of the sporulating leaves averaged only half that of the nonsporulating leaves. In the test of May 15, 12 cm. of leaf on systemically infected plants were harvested after 10 days and the weight of the sporulating leaves averaged only one-fourth that of the nonsporulating leaves. In all tests reported in table I, except one test of hop mildew, the reduction in green weight associated with sporulation was highly significant. That the injury from sporulation of hop mildew is less than that of onion mildew is in accordance with field observations, but severe sporulation injury from spinach mildew in the field, as indicated by these tests, has not been observed by the writer.

In 2 tests with onion and 2 with hop, healthy leaves or plants were subjected to the same incubation period and treatment as the diseased plants. In these tests there was no apparent consistent effect of the incubation treatment on the green weight of healthy tissues. The results of table 1, therefore, can fairly safely be attributed to the sporulation of the fungus and not to some direct environmental effect.

In addition to the evidence of sporulation injury presented in table 1, indirect evidence is believed to be apparent in field data. In the 1938 tests of fungicides for the control of onion mildew at Milpitas, applications of rosin-lime-sulphur and malacite-green produced increases in yield (Table 2) that are apparently out of proportion to the effect of these sprays in reducing infection. For example, fortnightly applications of rosin-lime-sulphur and of cuprocide resulted in about equal downy-mildew infection as of May 24, but the rosin-lime-sulphur plots outyielded the cuprocide plots by about 8 times. The writer believes these differences in yield may

TABLE 1.—*Magnitude of sporulation injury with some downy mildews*

Host and date of inoculation	Time, days		Number of paired units	Average green weight, grams		Percentage reduction in green weight associated with sporulation[a]
	Inoculation to incubation	Incubation to harvest		Control, sporulation prevented	Sporulation induced	
Onion						
April 15, 1938	15	18	11	4.43 per pot	2.05 per pot	54 ± 5 H.S.[b]
April 15, 1938	15	18	11	0.155 per plant	0.104 per plant	33 ± 7 H.S.
May 21, 1938	13	5	6	0.40 per leaf	0.21 per leaf	47 ± 10 H.S.
May 15, 1939	9	10	4	0.57 per leaf	0.14 per leaf	75 ± 11 H.S.
Hop						
May 26, 1938	7	4	12	0.59 per leaf	0.53 per leaf	10 ± 3 H.S.
May 26, 1938	7	4	4	0.42 per leaf[c]	0.32 per leaf	24 ± 12
Spinach						
Oct. 1, 1938	5	2	5	1.86 per pot	0.96 per pot	48 ± 8 H.S.

[a] Mean and standard error, calculated independently for each test.

[b] Highly significant, odds 99:1 or greater.

[c] In this test sporulation was prevented by exposing the leaves overnight to vapors from 10 per cent potassium sulphide.

be attributable in part to the rosin-lime-sulphur and malachite-green in inhibiting sporulation of onion mildew, while the effect of cuprocide on sporulation was less pronounced. This evidence is, of course, not conclusive.

TABLE 2.—*Fungicidal control of onion downy mildew. Milpitas, California, 1938. Yellow Bermuda. Randomized plots 10 feet long. Applications February 25; March 4, 14, 21, 30; April 6, 13, 20, 27; May 6, 16, 23*

Treatment	Number of plots	Average results per plot		
		Erect seed stalks April 27	Per cent seed stalks infected May 24	Grams cleaned seed
Control	10	68	59	2.67
2% rosin-lime-sulphur weekly	4	90	25	100.7
2% rosin-lime-sulphur fortnightly	4	88	29	39.2
0.25% cuprocide + CSO[a] weekly	3	96	19	46.2
0.25% cuprocide + CSO fortnightly	2	66	29	4.32
0.2% malachite-green + SS3[b] weekly	2	91	20	38.9
0.2% malachite-green + SS3 fortnightly	2	78	45	9.29

[a] 0.25 per cent cottonseed oil, emulsified with egg, was used as a supplement for cuprocide.

[b] 0.2 per cent Grasselli spreader (SS3) was used as a supplement for malachite-green.

EFFECT OF DOWNY-MILDEW SPORULATION ON WATER LOSS

To determine the effect of downy-mildew sporulation on water loss, paired samples of single detached leaves were placed with their bases in cotton-stoppered vials of water. One member of each pair was incubated overnight in moist chambers to induce sporulation, while the other was held in a dry environment. After sporulation the progress of water loss of both members of the pairs held in the same environment for several hours was determined by periodic weighings. The results of 4 such tests with onion and 4 with hop have failed to give consistent results. In the onion tests, with 5 to 7 paired units in each test, the water loss of the sporulating leaves was significantly higher than that of the controls in 2 tests, significantly lower than the controls in 1 test, and not significantly different in 1 test. In the tests with hop with 3 to 6 pairs of leaves per test, the water loss of the sporulating leaves was significantly higher than the controls in 1 test and not significantly different in 3 tests. From the above information it is, therefore, impossible to come to any definite conclusion as to the general effect of sporulation on water loss. It appears also that some factor other than sporulation may have been responsible for the differences observed, but exposure of healthy leaves to the same environmental manipulations in 3 tests failed to have any consistent effect on the water loss of the leaves. The tests of water loss were not arranged to compare properly the water loss of non-sporulating mildewed leaves with that of healthy leaves, but a superficial examination of the results of 4 tests in which the transpiration of healthy leaves was followed simultaneously with that of mildewed leaves did not indicate a marked difference.

EFFECT OF DOWNY-MILDEW INFECTION AND SPORULATION ON RESPIRATION OF ONION LEAVES

To measure the effect of downy-mildew sporulation on respiration, 2 g. green weight of onion leaf pieces were placed in 350 cc. rubber-sealed chambers with 10 cc. of 0.1 N KOH at 16° C. in the dark. After an interval of 14 to 23 hours the KOH solution was titrated with HCl and the amount of CO_2 absorbed was calculated. From this was subtracted the amount of CO_2 absorbed by a KOH blank containing no leaves and the CO_2 of respiration was determined. The dry weight of the leaves was ascertained and the results are expressed in table 3 as milligrams of CO_2 produced per gram dry weight of leaves per hour. In the first 2 tests leaves of greenhouse plants were used subsequent to sporulation. In the last 2 tests leaves that had not previously sporulated were used, and sporulation was prevented on the nonsporulating leaves by adding a drop of benzene to the appropriate containers, while sporulation occurred during the respiratory period on the nontreated leaves. A few cc. of dilute lime-sulphur were as effective as benzene in preventing the sporulation of onion or hop downy mildew in

TABLE 3.—*Effect of mildew sporulation on respiration of excised onion leaves, 1939*

Date of test	Number of replications	Hours in respiration chamber	Milligrams CO_2 per gram dry weight of leaf tissue per hour			
			Healthy	Mildewed, not sporulating	Mildewed, sporulating	Sporulating leaves from which sporophores were removed
Oct. 3[a]	2	23	1.84	2.14	2.32	....
Oct. 7[b]	4	22	1.44	2.32	1.86	1.79
Oct. 21[c]	2	14	0.91	....	1.96	....
Nov. 1[d]	2	17	0.96[e]	1.79	1.38	....
Nov. 8[f]	3	18	0.94[g]	1.80	1.65	....
Average[h]	11 (total)	20	1.29	2.01	1.80	....

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[a] Locally infected leaves that had sporulated the previous night, placed in respiration chambers at 3 p.m. Healthy, mildewed nonsporulating, and mildewed sporulating leaves of same age and culture.

[b] Systemically infected leaves that had sporulated the previous night, placed in respiration chambers at 9 a.m.

[c] Systemically infected leaves excised at 9: 30 p.m. and placed in respiration chambers. Sporulation of mildewed leaves was luxuriant under these conditions.

[d] Locally infected leaves excised at 9 p.m. and placed in respiration chambers. 1 drop benzene in 350 cc. respiration chamber used to prevent sporulation.

[e] Healthy leaves with benzene in chamber were uninjured and respired 1.0 mg. CO_2 under similar conditions.

[f] Systemically infected leaves excised 9 p.m. and placed in respiration chambers. 1 drop of benzene added to respiration chambers to prevent sporulation.

[g] Healthy leaves with benzene respired 0.95 mg. CO_2.

[h] Oct. 21 data omitted because no test of mildewed nonsporulating leaves was included.

Increase in respiration due to infection (without sporulation) was 56 ± 13 per cent of respiration of healthy leaves (calculated from 11 above paired samples). Increase highly significant.

Decrease in respiration of mildewed leaves due to sporulation was 10 ± 4.6 per cent of respiration of nonsporulating (calculated from above 11 paired samples). Decrease significant.

closed chambers, but could not be used in these tests because the KOH used to absorb the CO_2 also absorbed the H_2S from the lime-sulphur and thereby reduced the effectiveness of the lime-sulphur in preventing sporulation. Although heavier dosages of benzene were definitely injurious, the 1 drop of benzene used in these tests caused no marked injury to the leaves and had no apparent effect on the respiration of healthy leaves. Units of healthy leaves were included in all tests and in one test the respiration of mildewed sporulating leaves from which the sporophores were removed by rubbing the leaves with cotton was followed simultaneously with the other treatments.

The respiration of mildewed leaves averaged 69 per cent higher than that of healthy leaves, with the difference highly significant. The respiration of sporulating leaves was 10 per cent less than that of nonsporulating leaves, a surprising effect, and the difference was significant. The respiration of mildewed leaves from which the sporophores were removed was 4 per cent less than that of sporulating leaves from which the sporophores were not removed; the difference was not significant (only 4 determinations).

THE CAUSE OF SPORULATION INJURY

The foregoing tests of the effect of downy mildew on transpiration and respiration indicate to the writer that these effects are insufficient to account for the marked injury associated with downy-mildew sporulation. A more likely explanation is considered to be the exhaustion of nutrients in the leaf and possible formation of toxins in the process of sporulation. Some surface area, stomatal and weight relations of sporulating onion leaves were, therefore, studied. Table 4 presents the averages of 2 sets of determinations on systemically infected leaves which had sporulated for the first time. The most significant item in regard to sporulation injury, recorded in this table, is that 5 per cent of the dry weight of the infected leaf area may be transformed to sporangiophores and sporangia in the course of a single night of sporulation. When it is considered that a

TABLE 4.—*Surface area and stomatal and weight relations of sporulating downy mildew on an onion leaf*

Number of stomata per cm.^{2} of mildewed leaf surface	10,980
Number of sporangiophores per cm.^{2}	1,560
Number of sporangiophores per occupied stoma	1.42
Percentage of stomata occupied by sporangiophores	10 per cent
Number of sporangia per sporangiophore	25
Number of sporangia per cm.^{2} of leaf	39,100
Surface area of sporangia per cm.^{2} of leaf	0.14 cm.^{2}
Surface area of sporangiophores per cm.^{2} of leaf	0.76 cm.^{2}
Green weight per cm.^{2} of mildewed leaf	0.0279 g.
Dry weight per cm.^{2} of mildewed leaf	0.00226 g.
Dry weight of sporangia + sporangiophores per cm.^{2} of leaf	0.00012 g.
Sporangia + sporangiophores as % of dry weight of leaf	5.3 per cent[a]

[a] Average of 6 determinations.

given area of leaf may sporulate nightly until it is exhausted and dies (has been observed for 10 consecutive nights), and that the sporophores and sporangia are probably formed from labile constituents of the leaf, such as those in the cell sap, it is not surprising that the injury from sporulation may be so great.

Other items of table 4 may bear a relation to sporulation injury. For instance, about 10 per cent of the stomata may be occupied by sporangiophores in a single night of sporulation. Moreover, the substomatal cavities of unoccupied stomatal openings may be occupied by protoplasm-rich mycelium, presumably sporangiophore initials for a later sporulation. If it be considered that transpiration is mainly stomatal, the plugging of the stomatal openings and substomatal cavities by sporangiophores and sporangiophore initials might be a factor in reducing transpiration. On the other hand, if transpiration and respiration occur actively in the sporophores of the fungus it would be expected that sporulation might greatly increase the activity of these processes for the host parasite complex, since the surface area of the host fungus combination is almost doubled by the sporulation process. Another possibility is that the exhaustion of labile constituents from the host cells in the process of sporulation might be responsible for a reduction in the respiration of these cells. A likely possibility of sporulation injury, which was not investigated, was the effect on photosynthesis. Photosynthesis might be reduced because of the general exhausting effect of sporulation on the host and because of the shading effect of sporangiophores and sporangia. There are other possibilities.

SUMMARY

Sporulation injury by the downy mildews of onion, hop, and spinach was followed by comparing the green weight of tissues on which sporulation had occurred with that of similar tissues on which sporulation was prevented. The green-weight reduction due to sporulation averaged 55 per cent of the green weight of healthy leaves in 3 tests with onion, 17 per cent in 2 tests with hop, and 48 per cent in 1 test with spinach. In field plots of infected onion plants yield increases from the use of rosin-lime-sulphur and malachite-green sprays, which tend to inhibit the sporulation of onion downy mildew, were greater than from the use of a cuprocide spray, which has less effect on sporulation.

Attempts to determine whether sporulation had any effect on water loss of infected onion and hop leaves indicated increased water loss in some tests and decreased water loss in others. The respiration of nonsporulating mildewed leaves was 56 per cent higher than that of healthy and the respiration of sporulating leaves was 10 per cent less than that of nonsporulating infected leaves.

On heavily sporulating onion leaves about 10 per cent of the stomata were occupied by sporangiophores, and the surface area of the sporangio-

phores plus sporangia was approximately equal to the area of the leaf surface. The dry-matter content of the sporangiophores plus sporangia produced during the night of sporulation amounted to 5 per cent of the dry weight of infected leaves; and this transfer of host nutrients to the fungus is believed to be the feature of sporulation most likely responsible for the injury observed.

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EFFECT OF ROOT-KNOT NEMATODE ON TOMATO WILT[1]

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The root-knot nematode, *Heterodera marioni* (Cornu) Goodey, has been suspected of lowering the resistance of tomatoes to the wilt fungus, *Fusarium lycopersici* Sacc. Young[2] tested many tomato varieties and found that wilt resistance was nearly always lessened where the root-knot nematode was abundant. In the case of cotton, the reaction of several varieties grown in soil infested with both the fusarium wilt fungus and the root-knot nematode is described by Miles.[3]

Further evidence on this subject was found in a field near Hope, Texas, in the Yoakum-Hallettsville tomato belt in 1940. Although the field was set with wilt-resistant Marglobe tomatoes, large sections of the field were almost a total loss. However, other sections of this field produced a fair crop of tomatoes and the differences were distinct to the row (Fig. 1). Apparently, *Fusarium lycopersici* killed the plants, although a distinct correlation between the number of root knots and the severity of tomato wilt also was noted.

According to the owner of the crop, the history of the field was as follows: Spring crops of tomatoes in 1937 and 1938 had been followed by summer crops of peanuts. In 1939, after a spring crop of tomatoes, one part of the field was planted again to peanuts, while a second was planted to cowpeas, and a third to a mixture of cowpeas and watermelons. In 1940, tomato wilt caused almost a total loss following Little Clay and Whipporwill cowpeas, severe damage following Blackeye cowpeas (somewhat resistant to root-knot) and the combination of cowpeas and watermelons. Only moderate damage, however, followed the planting of peanuts (Table 1). Most of the tomatoes in the field were harvested in the green-wrap stage from May 20 to 25. The different sections were photographed on May 27.

Table 1 and figure 1 illustrate the prominent differences in the preva-

TABLE 1.—*Effect of crops susceptible and resistant to root knot on the severity of tomato wilt*

Summer crop 1939	Susceptibility to root-knot	Percentage tomatoes wilted—1940	
		May 9	May 27
Peanuts	Very resistant	7.5	33.5
Blackeye cowpeas	Somewhat susceptible	47.5	85.0
Little Clay cowpeas	Very susceptible	65.5	95.5
Whippoorwill cowpeas	Very susceptible	68.0	94.5
Watermelons and cowpeas	Both susceptible	29.0	80.5

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[1] Published with the approval of the Director of the Texas Agricultural Experiment Station as Technical Paper No. 630.

[2] Young, P. A. Tomato wilt resistance and its decrease by *Heterodera marioni*. Phytopath. 29: 871–879. 1939.

[3] Miles, L. E. Some tests of varietal susceptibility to a combination of root-knot nematode and cotton wilt. Phytopath. 29: 974–978. 1939.

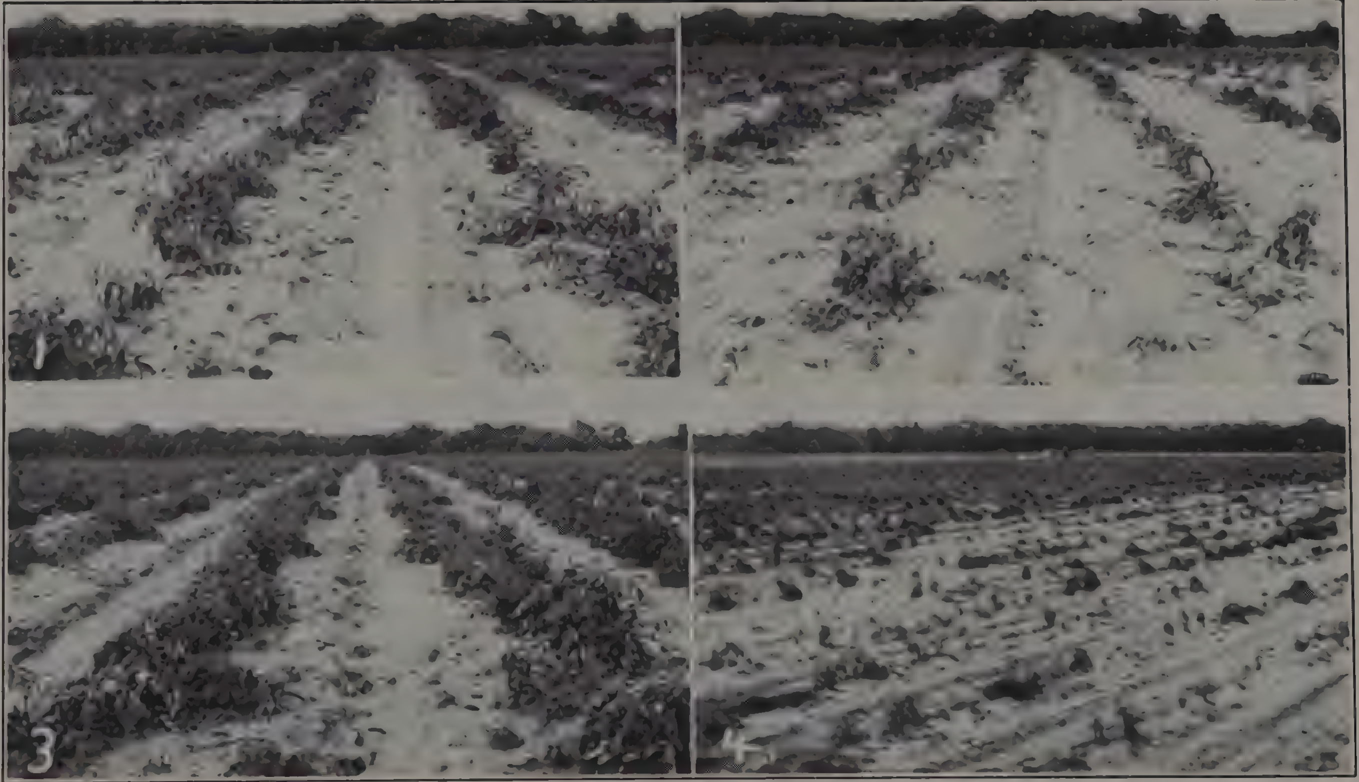

FIG. 1. Sections of tomato field, where both the root knot nematode and fusarium wilt were present, showing effects of previous crops upon the incidence of wilt: (1) following Blackeye cowpeas; (2) following Little Clay cowpeas; (3) following peanuts; and (4) general view of field with plots 2, 1, and 3 in that order from the front to background.

lence of tomato wilt in the different sections of the above field. Differences in the nematode population apparently is the reason for these differences. Godfrey[4] showed a correlation between the number of root galls and the soil population of *Heterodera marioni*. In this case, the largest number of root knots on tomatoes was found following Little Clay and Whipporwill cowpeas, fewer after Blackeye cowpeas, and the smallest number in land following peanuts. These data add to the evidence that root knot decreases the wilt resistance of the Marglobe tomato.

TABLE 2.—*Effects of root-knot nematode infection on susceptibility of tomatoes to fusarium wilt*

Variety	Weighted percentage of wilt resistance				Decrease by root-knot infection	
	1939		1940		1939	1940
	A[a]	B[b]	A	B		
					Per cent	Per cent
Blair Forcing	68	60	75	68	8	7
Bonny Best	......	......	15	16	......	0
Bohn-Tucker T463[c]	......	......	100	100	......	0
Break O'Day	......	......	65	50	......	15
Buckeye State	......	......	83	64	......	19
Early Baltimore T280	46	40	......	......	6	......
Early Baltimore T353	57	53	......	......	4	......
Grothen's Red Globe	67	49	70	47	18	23
Grothen's Red Globe T319	75	62	......	......	13	......
Grothen's Red Globe T320	62	53	......	......	9	......
Gulf State Market	35	26	48	29	9	19
Illinois Baltimore	71	59	76	53	12	23
Illinois Pride	72	68	......	......	4	......
Long Calyx Forcing	62	55	......	......	7	......
Louisiana Pink T346	83	76	89	66	7	23
Louisiana Pink T324	77	65	......	......	12	......
Louisiana Red	76	68	83	61	8	22
Louisiana Red T385	72	63	......	......	9	......
Marglobe	57	42	75	54	15	21
Marglobe T1B	......	......	77	39	. .	38
Marhio	59	34	......	......	25	......
Marvana	41	25	......	......	16	......
Marvel	70	62	......	......	8	......
Michigan State	54	47	74	50	7	24
Michigan State T356	60	49	......	......	11	......
Norton T289	64	55	......	......	9	......
Prairiana	58	37	60	49	21	11
Pritchard	56	50	65	51	6	14
Riverside	71	58	......	......	13	......
Riverside T314	74	62	......	......	12	......
Rutgers	65	43	74	41	22	33
Rutgers T317	74	49	......	......	25	......
Rutgers T382	68	52	......	......	16	......
Stone	14	12	34	14	2	20
Sureset Forcing	63	54	......	......	9	......
White-flowered T337	61	48	70	55	13	15

[a] Field practically free from *Heteradora marioni*, but infested with *Fusarium lycopersici*.

[b] Field badly infested with both *H. marioni* and *F. lycopersici*.

[c] Numbers preceded by T represent Texas Station selections.

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[4] Godfrey, G. H. Indicator plants for measuring soil populations of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Soil Science 38: 3–27. 1934.

Additional information on the decrease of tomato-wilt resistance by root knot was secured by the junior writer in 1939 and 1940 at Jacksonville, Texas, using the same fields, methods of culture, and method of calculating wilt resistance as described previously.[5] The two red-soil (reclassified as Ruston) fields, designated as A in table 2, retained their high degree of infestation with *Fusarium lycopersici,* and the trace of root knot that appeared on tomatoes in parts of these fields in 1940 was considered negligible. The comparative field, listed as B in table 2, of Norfolk fine, sandy-loam soil, which retained a high degree of infestation with both *F. lycopersici* and *Heterodera marioni* was used. Only the wilt-immune tomato strains were not killed by fusarium wilt in these fields.

The data from nearly 7000 tomato plants, averaging 23 plants per plot, are given in table 2. More plots were used for testing Break O'Day, Grothen's Red Globe, Gulf State Market, Illinois Baltimore, Marglobe, Pritchard, and Rutgers, than for most of the other varieties. Bohn-Tucker hybrid selection No. 463 remained immune from fusarium wilt in the sandy field even when most of main roots were badly infested with *Heterodera marioni.* The slight wilt resistance of the Bonny Best tomato was not further decreased by root knot, behavior common to varieties very susceptible to fusarium wilt. However, the Stone variety, in 1940, showed a large decrease in wilt resistance because of root knot. Nearly all varieties described in table 2 showed a serious decrease in wilt resistance, apparently because of root-knot-nematode infection, and 30 of the tests showed decreases of 11 to 38 per cent in resistance to fusarium wilt.

SUMMARY

The loss from tomato wilt was observed to be reduced in a commercial field where root-knot-resistant peanuts were grown in the crop rotation. In contrast, almost total loss (presumably attributable to tomato wilt) resulted when the tomato crop followed root-knot-susceptible varieties of cowpeas. The root-knot nematode population was largest following crops susceptible to root knot. Further evidence is presented to show that the root-knot nematode reduces the wilt resistance of many varieties of tomatoes. A wilt-immune selection of tomatoes retained its immunity despite abundant root knot.

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[5] See footnote 2.

VARIATIONS IN PHYTOMONAS VESICATORIA

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In a recent article from Connecticut, Horsfall and Donnell (4) note considerable differences in varietal susceptibility of peppers to *Phytomonas vesicatoria* (Doidge) Bergey *et al.* They also comment on the fact that Weber (5) in Florida found no differences among 10 varieties of pepper and that Bullnose, one of their resistant varieties, "appears to react differently in the South than in the North." In an oral communication Charles Chupp made the statement to the writers that in New York State the disease caused by *Phyt. vesicatoria* has been prevalent on peppers on Long Island but not on tomatoes, and, in upstate New York, it has been found on tomatoes but not on peppers. The disease has been reported prevalent on both tomatoes and peppers in Florida and in other parts of the South.

Certain recent work by the writers, while not extensive in scope, does bring to light certain hitherto unknown characteristics of this pathogen and possibly explains the reported discrepancy in varietal susceptibility of peppers just mentioned and also the reason for the pathogen's appearance on only one host in a locality.

At the beginning of the investigation here reported, 10 isolates of *Phytomonas vesicatoria* were on hand and were used during the experiments. Seven of these were from pepper; 1 from a diseased pepper leaf collected in Vermont, 1937; 3 from material sent by H. S. Cunningham from Long Island, 1939; and 3 from specimens from A. H. Eddins, Hastings, Florida, 1939. There were also 3 isolates from tomato; 1 from Vermont, 1937; 1 from New York, 1937; and a culture received from Mary K. Bryan, a number of years ago. Later, R. E. Lincoln of Purdue University, sent the writers an isolate from tomato and a culture of *Phyt. vesicatoria* var. *raphani* (White) Burkh.

Inoculation experiments were conducted in the greenhouses during the winter of 1939 to 1940 with these 10 isolates of *Phytomonas vesicatoria* on some 8 or 9 varieties of both peppers and tomatoes. Leaves were used in the main for comparison of severity of infection since a sufficient number of fruits were not available.

The following varieties of tomatoes were inoculated: Greater Baltimore, John Bear, Bonney Best, Marglobe, Rutgers, Pritchard, Harris' Extra Early, Nystate, and Ponderosa. The Vermont pepper strain of the pathogen produced no infection on the tomato, although the 3 Long Island pepper isolates caused a very few slight lesions. The pepper strains from Florida produced good infection on the tomato leaves, as did the 3 tomato isolates.

Some tetraploid tomato plants, growing in the greenhouse for other purposes, were inoculated at this time with the Vermont pepper isolate and 2 tomato isolates. Only the latter 2 caused infection.

The picture on the pepper varieties was somewhat different and equally interesting. The following varieties of peppers were inoculated with the various isolates of *Phytomonas vesicatoria:* Bullnose, Chinese Giant, Oshkosh, Harris' Early Giant, World Beater, California Wonder, and Ruby King. The pepper isolates of the pathogen from Long Island and from Florida produced good infection on all the varieties except Oshkosh and Harris' Early Giant. On these two varieties infection was very light. The pepper isolate from Vermont and the 3 tomato isolates produced good infection on all the varieties of peppers, but the lesions formed slowly.

The conditions under which the plants were inoculated were the same throughout, and the pepper and the tomato varieties respectively were inoculated at the same time. In examining the data the results could not be interpreted on the age of the isolates.

From these infection experiments it appeared that the Vermont pepper isolate was similar to the Long Island pepper isolates and these were distinct from the isolates from tomato. The 3 Florida strains from pepper appeared to be intermediate.

After determining the pathological differences among the strains of the organism, tests were made to determine whether there were any physiological differences in culture. In appearances, on agar, all isolates were similar. They also behaved similarly in utilizing nitrates, although they did not reduce them to nitrites. None of them utilized asparagine, tyrosine, or hippuric acid. They all liquefied gelatin and did not produce indol. In milk, the pepper isolates reduced litmus and methylene blue more rapidly than did the tomato isolates and they also produced more H_2S. These differences in degree of characteristics, however, could scarcely be used for a decisive separation of the organisms. Another character, nevertheless, which was correlated with these slight differences, was that of the ability of the various pathogens to hydrolyze starch. None of the pepper isolates had this ability, but the isolates from tomato did, including the one from R. E. Lincoln and the variety *Phytomonas vesicatoria* var. *raphani.* The difference was very clear-cut and distinctive. The method used for determination of this character was the starch-agar iodine test. As far as the writers are aware, this difference in the characteristics of the pathogen from the two hosts has not been reported before, although search through literature revealed the following points: Higgins (3), in describing the pathogen from pepper, states that starch hydrolysis is feeble; he does not, however, give his methods of determination. Gardner and Kendrick (2), using tomato strains found a ''strong diastatic action,'' and likewise Miss Doidge (1) reports diastatic action by the tomato pathogen. It is probable that this difference between the pepper and tomato pathogens is widespread. If such be the case, one might gather that, while it is possible that certain of these strains of *Phyt. vesicatoria* have the ability to cross-infect, in nature they are, as a rule, not promiscuous. No work has been done as yet to determine whether the passage through pepper or tomato might have an influence on the pathogen, through selection or otherwise, to bring out or to suppress this ability to hydrolyze starch. Also, what part hydrolysis of starch plays in the pathogenicity of the organism cannot be ascertained and the writers are of the opinion that it is merely a correlated character.

The bearing of these findings concerning *Phytomonas vesicatoria* is mainly on the selection and breeding of disease-resistant strains of pepper and tomato. In this paper it is pointed out that certain races of the pathogen do exist. The question of how many exist is problematical. All breeding work will have to consider these questions. From a taxonomic standpoint the writers do not feel that the differences in the strain are sufficiently great to propose varietal names. Furthermore, before such names are given, one should be sure of the stability of the characters on which the separations are made.

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LITERATURE CITED

1. DOIDGE, E. M. A tomato canker. Ann. Appl. Biol. 7: 407–430. 1921.
2. GARDNER, M. W., and J. B. KENDRICK. Bacterial spot of tomato. Jour. Agr. Res. [U.S.] 21: 123–156. 1921.
3. HIGGINS, B. B. The bacterial spot of pepper. Phytopath. 12: 501–516. 1922.
4. HORSFALL, J. G., and A. D. DONNELL. Varietal susceptibility of peppers to bacterial spot. The Plant Dis. Rptr. 24: 34–36. 1940.
5. WEBER, G. F. Diseases of peppers in Florida. Fla. Agr. Exp. Stat. Bull. 244: 6–9. 1932.

PHYTOPATHOLOGICAL NOTES

Classification of Hawaiian Commelina-mosaic Virus.—A mosaic disease of *Commelina nudiflora* L. has been recognized for many years in Hawaii. Kunkel[1] mentions it as early as 1921. The disease and its causal agent have, however, been relatively little studied. In 1935, Carter[2] transmitted commelina mosaic to pineapple, *Ananas comosus* Merr., by mechanical inoculation and, in 1937, he[3] demonstrated its transmissibility to *C. nudiflora* and to pineapple by means of the aphids *Aphis gossypii* Glover, *Myzus persicae* (Sulz.), and *Macrosiphum solanifolii* (Ashm.). The symptoms of Hawaiian commelina mosaic in *C. nudiflora* are similar to those produced by the southern celery-mosaic strain[4] of cucumber-mosaic virus, *Marmor cucumeris* H. var. *commelinae* H., in the same host plant as described by Doolittle[5] and Doolittle and Wellman.[6] Furthermore, the two viruses are transmitted by the same species of aphids. It thus seems likely that they are closely related. Definite proof of this relationship has not, however, been available. Recently, the writer found that Hawaiian commelina-mosaic virus protects zinnia, *Zinnia elegans* Jacq., from infection with the indicator strain of cucumber-mosaic virus, *Marmor cucumeris* H. var. *judicis* H., thus demonstrating a close relationship between these two viruses. It is the purpose of this note to present the evidence on which this statement is based.

Dr. Walter Carter of the Hawaiian Sugar Planters Association was kind enough to supply leaves of *Commelina nudiflora* infected with a yellow strain of mosaic and others infected with a green strain. The symptoms of these two strains in commelina differed in that the yellow strain produced a brilliant-yellow mottling on a dark-green background, whereas the green strain produced a light-green or pale-yellow mottling. The experiments with these viruses were conducted in a greenhouse where every precaution was taken, including fumigation at frequent intervals, to prevent accidental spread of the viruses. The yellow strain of virus was readily transmitted to dicotyledonous hosts by means of rubbing, but difficulty was experienced with the green strain. On the second trial, however, infection was obtained with the latter in Turkish tobacco, *Nicotiana tabacum* L. It was relatively easy to transmit the virus from tobacco to other plants of this species or to the other host plants tested. Infection was obtained with both strains in the following species: *N. glutinosa* L., *N. alata* Link and Otto, *N. bigelovii*

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[1] Kunkel, L. O. A possible causative agent for the mosaic disease of corn. Bull. Exp. Sta. Hawaiian Sugar Plant. Assoc., Bot. Ser., 3: 44–58. 1921.

[2] Carter, Walter. Mechanical transmission of two viruses to pineapple. (Abstract) Phytopath. 25: 10. 1935.

[3] Carter, Walter. Aphis transmittal of *Commelina nudiflora* L. mosaic to pineapple. Am. Entom. Soc. Amer. 30: 155–158. 1937.

[4] Price, W. C. Classification of southern celery-mosaic virus. Phytopath. 25: 947–954. 1935.

[5] Doolittle, S. P. *Commelina nudiflora,* a monocotyledonous host of celery mosaic. Phytopath. 21: 114–115. 1931.

[6] Doolittle, S. P., and F. L. Wellman. *Commelina nudiflora,* a monocotyledonous host of a celery mosaic in Florida. Phytopath. 24: 48–61. 1934.

Wats. var. *quadrivalvis* Pursh., *N. glauca* Grah., *Cucumis sativus* L., and *Zinnia elegans*. In general, the symptoms were similar to but distinguishable from those of ordinary cucumber mosaic in the same hosts. Likewise, the symptoms were distinguishable from those of the southern celery-mosaic strain of cucumber-mosaic virus. The yellow strain of Hawaiian commelina-mosaic virus consistently produced more brilliant symptoms than the green strain.

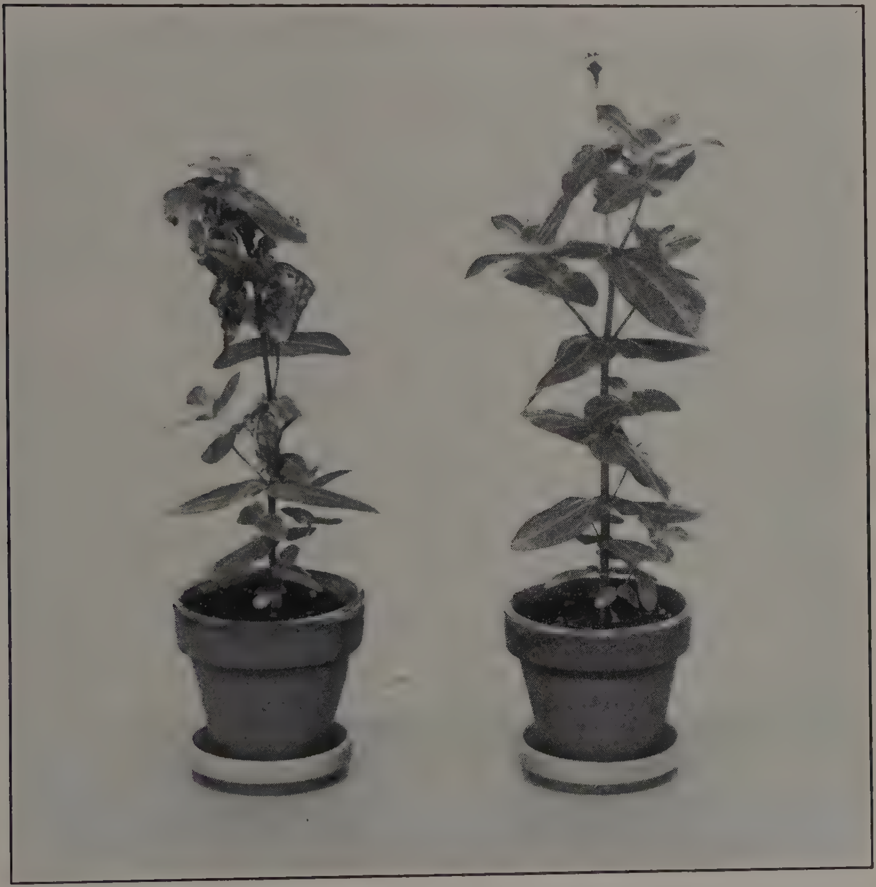

FIG. 1. Photograph of zinnia plants 14 days after inoculation with the indicator strain of cucumber-mosaic virus. Previous to inoculation, the plant on the left was healthy, while that on the right was affected by the yellow strain of Hawaiian commelina-mosaic virus, the symptoms of which were mild and do not show in the photograph. Commelina-mosaic virus has protected the plant from infection with the indicator strain of cucumber-mosaic virus. (Photograph by J. A. Carlile.)

The susceptibility of zinnia made it feasible to perform cross-protection tests similar to those previously described.[7] In the Golden Gem variety of zinnia, which was used in these tests, the yellow strain caused a green mottling disease, while the green strain caused only a mild transient mottling which soon disappeared. Since similar results were obtained with the yellow and green strains, only the results of a typical experiment with the yellow strain will be cited. On March 30, 1940, 12 young zinnia plants were inoculated with the yellow strain of commelina-mosaic virus. All 12 plants became infected and showed mottling symptoms. On April 19, 6 mottled leaves on each of the 12 infected plants and 6 comparable leaves on 12 healthy zinnias were rubbed with the indicator strain of cucumber-mosaic virus. On April 24, a total of approximately 3,600 necrotic lesions had developed on the inoculated leaves of the previously healthy plants, while not a single lesion developed on the inoculated leaves that were mottled by commelina-mosaic virus. Figure 1 illustrates the results of the experiment 14 days after inoculation with the indicator strain of cucumber-mosaic virus. These results, together with those of similar tests not reported here, demonstrate a close relationship between the Hawaiian commelina-mosaic virus strains and other viruses of the cucumber-mosaic virus group.

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[7] Price, W. C. Acquired immunity from cucumber mosaic in zinnia. Phytopath. 25: 776–789. 1935.

The writer has not observed Hawaiian commelina mosaic under field conditions. From personal communications, however, it appears that the symptoms of this disease take a diversity of forms, ranging from a very brilliant yellow mottling to a very mild green, almost indistinguishable, mottling. It thus seems apparent that commelina in Hawaii is affected by a number of different strains of cucumber-mosiac virus. None of these, including the two variants dealt with in this paper, have been sufficiently studied to be readily identified from a published description of their characteristics. For this reason, it seems best at this time not to apply a formal name to either of the two strains herein discussed but to refer to them as yellow and green variants of cucumber-mosaic virus.—W. C. PRICE, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Comparison of Susceptibility of the American Elm and Several Exotic Elms to Ceratostomella ulmi.—In Europe, extensive inoculations have been made to test the resistance of various species and varieties of elm to *Ceratostomella ulmi* Buisman.[1] Few reports, however, have appeared comparing the susceptibility of the American elm (*Ulmus americana* L.) with that of several exotic elm species growing in the United States. Some data were obtained from a series of inoculations of several comparable species and varieties of elm growing in an experimental nursery maintained by the Division of Forest Pathology, Bureau of Plant Industry, near Morristown, N. J. The trees ranged from $\frac{3}{8}$ to $1\frac{1}{4}$ in. in diameter at the base. All were seedlings, except the Christine Buisman elms, which represent a clone propagated by layering. The "Mormon elm" received under that name from one of the western States appears to be a type of one of the small-leaf European elms. The trees were inoculated from June 3 to 6, 1940. Inoculation was effected by making a vertical, tangential incision into the xylem with a $\frac{1}{8}$-in.

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[1] Went, J. C. Compilation of the investigations on the susceptibility of different elms to *Ceratostomella ulmi* Buisman in the Netherlands. Phytopath. Zeits. **11**: 181–201. 1938.

wood chisel and then immediately filling the wound with an aqueous suspension of spores of *C. ulmi*. Approximately 4 inoculations per diameter inch were made around the trunk of the tree near the base. These inoculations were considered to have subjected the trees to a severe test.

External symptoms of Dutch elm disease were apparent in all groups of trees 7 to 15 days after inoculation. Observations at various times during the growing season indicated that the population of *Ulmus americana* under test was being severely affected. *U. laevis* developed extensive wilting and dieback but seemed not to be affected so severely as *U. americana*. At the end of the growing season, the total main leader dieback on each tree was measured. The mean percentage of main-leader dieback was calculated for each species and variety, and the data analyzed statistically (Table 1).

TABLE 1.—*Comparison of infection and dieback of Ulmus americana and of several exotic species of elms inoculated with Ceratostomella ulmi in 1940*

Species	Number of trees inoculated	Percentage developing symptoms	Mean percentage of main leader dying back[a]
U. americana	100	100	90.89 ± 2.6810
U. laevis	88	100	81.09 ± 1.8452
U. foliacea	11	100	69.98 ± 8.8619
U. procera	28	100	53.02 ± 5.4720
U. japonica	10	100	23.73 ± 11.8960
"Mormon elm"	10	100	19.20 ± 1.3500
U. pumila	218	42	10.69 ± 0.8835
" " var. *pinnato-ramosa*	10	70	4.79 ± 0.9833
" " var. *arborea*	10	30	2.09 ± 0.7830
U. foliacea var. Christine Buisman	7	86	0.0

[a] Based on percentage of main leader dieback as of the end of the growing season.

The population of *U. americana* was more susceptible to *Ceratostomella ulmi* than that of any of the other elm species and varieties tested. Results for *U. japonica* should be considered indicative only, as the high standard error indicates too few individuals tested. The *pumila* group appeared rather resistant, and, as a whole, escaped serious injury from the disease. No main-leader dieback developed in any of the trees of the Christine Buisman selection of *U. foliacea*. Some of the trees, however, did lose several leaves, and, in a few cases, the terminal portion of one or two small twigs on lower side branches died; but, in every case, the dieback was negligible.

As a rule, the xylem discoloration associated with the disease was most pronounced and most extensive in the more susceptible species. The more resistant species or varieties, such as those of the *pumila* group and *Ulmus foliacea* var. Christine Buisman, usually showed fairly extensive invasion by the fungus, but fewer vessels were infected.—S. J. SMUCKER, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, N. J.

Isometric Crystals Produced by Pisum Virus 2 and Phaseolus Virus 2.[1]—The Trypan Blue technique for identification of viruses[2] has facilitated the finding of crystal phases in several plant viruses. The process has demonstrated, in *Vicia faba* infected with Pisum virus 2 and Phaseolus virus 2, crystalline entities that are of unusual interest because they occur in both the cytoplasm and nuclei of infected host cells. These crystals are from 0.3 to 4.0 μ in diameter and are plainly isometric. They stain deeply in Trypan Blue and can be shown, even in chlorenchymatous cells, but are more easily visible in epidermal cells, where they aggregate into crystalline viroplasts. A remarkable feature of their occurrence in nuclei is their frequent and consistent presence within the nucleoli. The nucleoli of diseased cells assume cubical shapes as they become filled with the isometric crystals. Usually 5 or more are present, but, occasionally, one large crystal fills the entire nucleolus. The presence of virus artifacts or inclusion bodies within nuclei is a common occurrence in virus-infected animal cells, but has been proved in only one other case in plants, namely, in *Nicotiana tabacum* infected with Nicotiana virus 7, as shown by Kassanis.[3] The fact that the crystalline inclusions, characteristic of each of these viruses, are plainly isometric suggests basic relationships between the 3 viruses. It has long been known that Pisum virus 2 and Phaseolus virus 2 are related because of close agreement between their physiological properties and host responses. Cytologically, they differ very little, the difference being that the crystals accompanying Phaseolus virus 2 are larger than those of Pisum virus 2 within the same host species (Fig. 1).

These isometric, isotropic inclusions may be considered comparable to the hexagonal crystals of tobacco mosaic that form within cells. The isometric crystals are more stable, however, and can be micro-dissected into physiological salt solution without being destroyed. The stability of the isometric crystals is such that they are retained in paraffin sections fixed in aceto-formalin and in whole mounts processed by the Dioxan technique.[4] They give the usual reactions for proteins. The crystals were not found in the foliar cells of healthy plants, but they were found in every case investigated where systemic invasions of either of these viruses induced mottling of leaves. They occur frequently within the guard cells of stomata. That these crystals are related to the causal agents is proved by the fact that they have been demonstrated in 4 different species of legumes inoculated with Pisum virus 2 and 2 species inoculated with Phaseolus virus 2. Their occur-

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[1] Published as Technical Paper No. 369 with the approval of the Director, Oregon Agricultural Experiment Station. Contribution of the Department of Botany in cooperation with the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] McWhorter, F. P. Separation of Tulip 1 virus from Lily-latent by cytological methods. Phytopath. 30: 788. 1940.

[3] Kassanis, B. Intracellular inclusions in virus-infected plants. Ann. Appl. Biol. 26: 705–709. 1939.

[4] McWhorter, F. P., and Elliot Weier. Possible uses of dioxan in botanical microtechnic. Stain Techn. 11: 107–117. 1936.

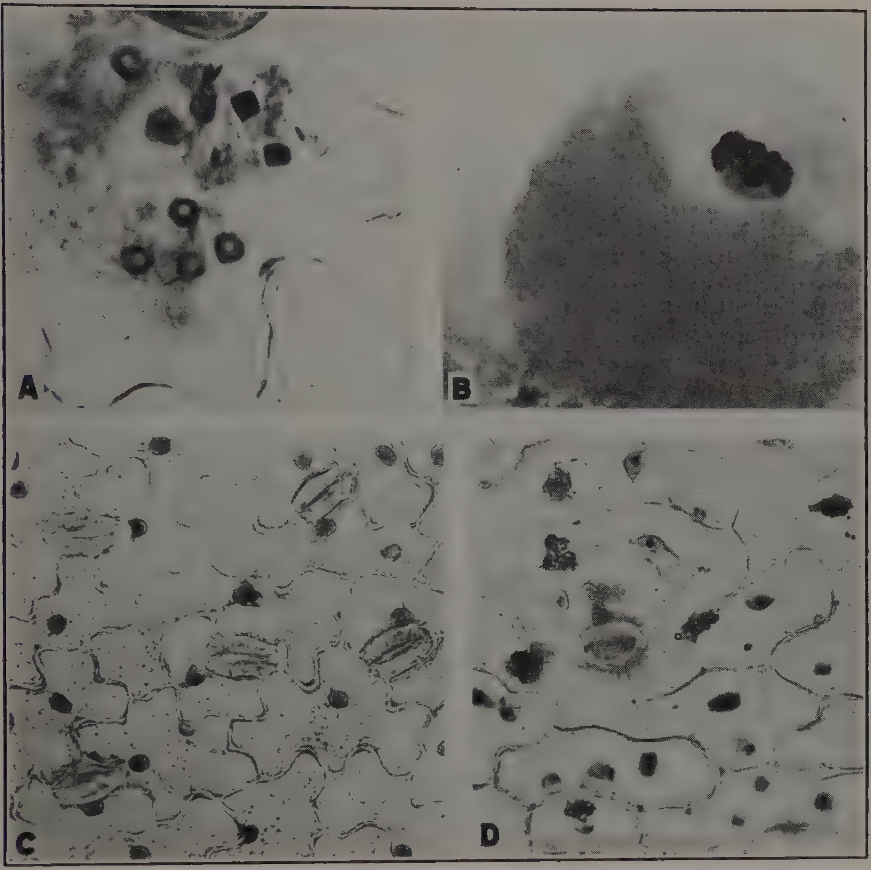

FIG. 1. Cytological evidence of pea virus 2 and bean virus 2 (Smith classification) in horse bean, *Vicia faba*. All preparations, including check, are whole mounts of leaf tissue treated with Trypan Blue. A. Isometric crystals accompanying bean virus 2 in cytoplasm. ×200. B. Portion of nucleus showing nucleolus filled with isometric crystals. This is from plant infected with bean virus 2. ×2000. C. Epidermal cells of healthy plant treated with Trypan Blue. ×200. D. Epidermal cells from leaf infected with pea virus 2. Note viroplasts in the form of crystalline reticules. ×200.

rence is, therefore, presumably a function of the virus rather than of the host.

Isometric crystals have not been found in legumes infected with Pisum virus 1, but this virus also causes structural changes in the nuclei of its hosts.[5]—FRANK P. MCWHORTER, Oregon Agricultural Experiment Station, Corvallis, Oregon.

Variation in Cultures of Certain Grass Smuts.[1]—Variants have been reported in a number of smut fungi[2] that attack crop plants belonging to the Gramineae. There is, however, very little reference in the literature to variation in smuts that attack grasses of lesser economic importance. Therefore, a preliminary study was made of the variability of some grass smuts that are found in Minnesota.

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[5] Acknowledgment is due Mr. Saul Rich, Research Assistant, Oregon Experiment Station, who isolated, plant tested, and subinoculated the viruses herein considered.

[1] Paper No. 1894 of the Scientific Journal Series, Minnesota Agr. Exp. Station. Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 65–1–71–140, sponsored by the University of Minnesota, 1940.

Monosporidial isolates[3] of *Ustilago sphaerogena* Burr., *Ustilago neglecta* Niessl., *Sphacelotheca panici-miliacei* (Pers.) Bubak., *Sorosporium syntherismae* (Pk.) Farl., and single promycelial cell isolates of *Ustilago crameri* Körn. were cultured in duplicate flasks on potato-dextrose agar and malt agar for about 6 weeks. Of the above smuts only *U. crameri* had been studied previously to any great extent in pure culture. In these studies the other 4 species were found to grow very well in culture. The chlamydospores germinated on potato-dextrose agar, producing promycelia with sporidia; the sporidia budded a few times then developed into a mycelial type of growth, except in the case of *U. sphaerogena,* which remained more or less sporidial.

The general appearance of the cultures of these grass smuts did not differ strikingly from any other smuts that have been grown in culture. Each of the smut species consisted of a number of cultural biotypes that differed from each other in such characters as color, topography, type of margin, consistency of colony, and direction and rate of growth.

The frequency of sectoring in the smuts studied is summarized in table 1. Many of the cultures sectored abundantly, while a few did not sector at all. Certain cultures sectored on potato-dextrose agar but not on malt agar and

TABLE 1.—*Frequency of sectoring of monosporidial isolates of certain grass smuts on potato-dextrose and malt agars*

Smut	Host[b]	Number isolates tested	Number isolates that sectored			Number sectors	
			Total	On p-d. a.	On malt agar	On p–d. a.	On malt agar
Ustilago sphaerogena	*Echinochloa crus-galli*	26	26	16	24	26	82
Ustilago neglecta	*Setaria lutescens*	25	20	13	13	32	51
Ustilago crameri[a]	*Setaria italica*	24	17	12	10	22	14
Sphacelotheca panici-miliacei	*Panicum miliaceum*	25	17	7	16	14	25
Sorosporium syntherismae	*Cenchrus* sp.	25	25	11	25	16	97

[a] Single promycelial cells.

[b] Nomenclature of grasses according to A. S. Hitchcock, Manual of the Grasses of the United States. (Washington). 1935.

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[2] Christensen, J. J. Physiologic specialization and genetics of the smut fungi. Bot. Rev. 6: 389–425. 1940.

[3] These cultures were isolated by Thomas Laskaris while he was in the Division of Plant Pathology at the University of Minnesota in 1938.

vice versa; generally, malt agar appeared to be more favorable for sectoring, except in the case of *Ustilago crameri.* Sectors differed from their parents in such characters as color, topography, type of margin, and direction and rate of growth. Many of the sectors were shown to be true variants since their cultural characters persisted and remained quite distinct from those of their respective parents on subsequent transfer to other flasks of agar.

From this study it is obvious that much the same type of variation occurs in these smuts as occurs in the smuts attacking graminaceous crop plants.—W. J. MARTIN and M. F. KERNKAMP, Department of Plant Pathology, University Farm, St. Paul, Minnesota.

Storage and Mounting of Demonstration Specimens.—The storage of demonstration specimens of various sizes presents a problem in laboratories of plant pathology, and especially of forest pathology, where many specimens are large and some are heavy or fragile. One solution of this problem, providing neatness and compactness, sturdiness, flexibility, and accessibility, is offered herewith. As shown in figure 1, it consists of a series of wooden drawers of such dimensions as to be interchangeable in wooden cabinets.

The cabinet is of 5-ply construction, without molding, baseboard, or other decorative superfluities, so that additional units can be grouped vertically or horizontally to meet expanding storage requirements or varying wall or floor space. In order to avoid waste spaces within the cabinet and enable ready movement of drawers when the door is open to 90 degrees, casing is omitted, and the door swings free of the interior. This is accomplished without outside hinges and without waste space between laterally adjacent cabinets either by cutting off the hinged edge of the door in line with the inner side of the hinged side wall of the cabinet and fixing the hinges inside (Fig. 1, (1)) or preferably by rounding the corners of the hinged edge of the door and pivoting the door on 2 pins extending into the upper and lower edges of the door from the lower side of the projecting cabinet top and the upper side of the projecting cabinet bottom, respectively (Fig. 1, (2)). By hinging the doors of laterally adjacent cabinets on opposite sides, a single long-shank padlock secures two doors. The sidewalls of the cabinet are routed on the inside at 4″ intervals to form horizontal grooves $\frac{1}{2}$″ wide and $\frac{3}{8}$″ deep. The grooves support removable shelves or drawers.

The drawers are of 4 sizes, the smallest (a) being of such a size (4″ × 12″ × 17″) as to fit into a compartment of a standard steel herbarium case (where a few are used). The next size (b) is approximately twice as high ($8\frac{1}{2}$″). The third size (c) is approximately the same height as the smallest ($4\frac{1}{2}$″), but approximately twice as wide ($24\frac{1}{2}$″). The largest size (d) is approximately twice as high (9″) and twice as wide ($24\frac{1}{2}$″) as the smallest. The bottoms of the two double-width sizes (c and d) extend $\frac{3}{4}$″ beyond their side walls, enabling their support in the cabinet side-wall grooves. The bottoms of the single-width sizes (a and b) are flush with their side walls and rest on a removable double-width shelf supported by the cabinet side-wall grooves.

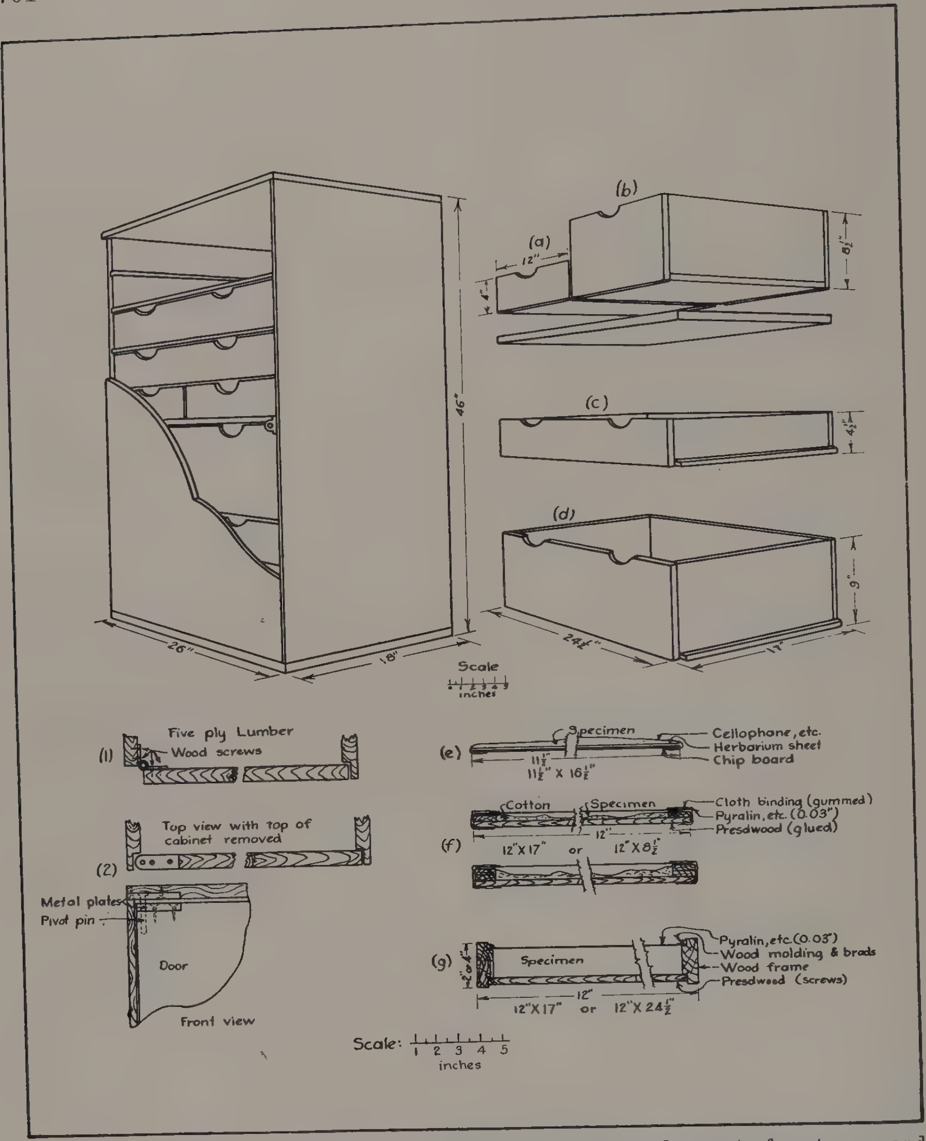
(a)
12"
4"
(b)
8½"
46"
(c)
4½"
(d)
9"
26"
18"
24½"
17"
Scale
inches
(1)
Five ply Lumber
Wood screws
(2)
Top view with top of cabinet removed
Metal plates
Pivot pin
Door
Front view
(e)
Specimen
Cellophane, etc.
Herbarium sheet
Chip board
11½"
11½" X 16½"
(f)
Cotton
Specimen
Cloth binding (gummed)
Pyralin, etc. (0.03")
Presdwood (glued)
12"
12"X17" or 12"X8½"
(g)
Specimen
2" or 4"
Pyralin, etc. (0.03")
Wood molding & brads
Wood frame
Presdwood (screws)
12"
12"X17" or 12"X24½"
Scale: 1 2 3 4 5
inches

FIG. 1. Cabinet, interchangeable drawers and shelves, and mounts for storage and display of demonstration specimens of various sizes; (1), (2)—methods of hinging cabinet door; (a–d)—drawers; (e–g)—mounts.

Ordinary specimens, either dried or in preservative liquid, are stored loose in the drawers. Rare or fragile specimens are mounted and placed directly on the removable shelves.

The methods of mounting described below are not new but have been found satisfactory. A flat herbaceous specimen is pasted in the usual manner on a standard, 11½″ × 16½″, herbarium mounting sheet, which is laid over a sheet of chip board for rigidity, and a larger flexible sheet of transparent plastic film (Cellophane, Kodapack, Lumarith, etc.) of 0.002″ thickness is laid over the specimen and pasted to the back of the board (e); if subject to rough handling, the edges of the mount may be bound with adhesive tape. Thicker specimens, not exceeding ½″, are laid on cotton batting on a rigid sheet of chip board, plywood, or preferably Presdwood, 12″ × 17″ or 8½″ × 12″, the sides raised by one or more glued strips of the same material, ¾″ wide; the whole is covered with a rigid sheet of transparent plastic film (*e.g.*, Pyralin), 0.030″ thick; and the edges of the mount are bound with gummed cloth binding tape (f). Thicker and larger specimens are laid on cotton or preferably screwed, wired, or glued to a sheet of plywood or preferably Presdwood screwed into one rabbeted edge of a wooden frame 12″ × 17″ or 17″ × 24½″, and 2″ or 4″ deep; and a sheet of glass, or preferably Pyralin, is fastened with brads and molding into the other rabbetted edge of the frame (g). A large specimen to be viewed on all sides is screwed to the frame and Pyralin is placed in both edges.—JOHN EHRLICH, School of Forestry, University of Idaho, Moscow, Idaho.

Evidence of Resistance in Alfalfa, Red Clover, and Sweetclover to Certain Fungus Parasites.—Observations in nurseries of alfalfa, red clover, and sweetclover at Madison, Wisconsin, in 1940, supplemented by some field observations, have produced evidence of resistance or differences in 9. susceptibility to ~~8~~ fungus parasites, most of which ordinarily are considered as of minor importance. In most cases evidence of such resistance appears to be new; but whether old or new, the immediate interest in such observations relates to the production of improved varieties in which uniform resistance, even to minor diseases, is sought and in which the introduction of high susceptibility to parasites previously considered minor should be avoided.

Three circumstances appear to have made possible these observations: first, climatic conditions were unusually favorable for fungus development, especially in the long and almost frost-free autumn; second, the diseases developed for the most part independently rather than simultaneously in the same nursery; and third, the nurseries contained diverse material in suitable plantings for the clear observation of plant characteristics.

ALFALFA

In alfalfa, a nursery of clonal lines and one of strains and varieties were especially favorable for the observations of plant differences that are usually obscured in the field.

Pseudopeziza medicaginis (*Lib.*) *Sacc.* Clonal lines showed striking differences ranging from almost complete freedom to defoliation with rapid development of apothecia on leaves. Plants with some resistance were found in all varieties represented in the nursery up to about 50 per cent in Ladak; but, in the field, only occasional plants of Ladak were found free from spots. A few of the clones showing differences in infection in the field have been inoculated with a pure culture in the greenhouse, where differences in disease development have been obtained corresponding to those in the field.

Peronospora trifoliorum de Bary. A few clonal lines showed uniformly distorted stems and superficial evidence of systemic infection, while some other lines had only a larger or smaller amount of leaf spotting. The majority of the clonal lines were not infected.

Stagonospora meliloti (Lasch) Petr. Leaf spotting from this was limited strictly to a few clones. When plants from these were dug in the autumn the crowns were more or less decayed.

Stemphylium botryosum Wall. In a nursery of varieties and strains differences ranging from light infestation to defoliation were recorded. Among the common varieties, Cossack and Grimm had significantly less injury than Ladak.

RED CLOVER

Inbred lines of red clover, resistant to powdery mildew (*Erysiphe polygoni* DC.), in the nursery showed clear differences in reaction to 3 other minor parasites.

Stemphylium sarcinaeforme (Cav.) Wilt. Clear-cut differences were evident between lines ranging from complete defoliation in some to a scarcity of lesions in others.

Uromyces trifolii fallens (Desm.) Arthur. Some susceptibility to rust was apparent in all lines, but, in a few, infection was so severe that leaves and stems of all plants were covered with uredial pustules.

Cymadothea trifolii Wolf. Marked resistance to sooty blotch was notable in most lines, but a few were so susceptible that the under surfaces of all leaves were completely blackened with stromata.

SWEETCLOVER

Pseudopeziza meliloti Syd. This leafspot was observed only in fields. In one field of white sweetclover, heavily infected in the first year, occasional plants free from the spot were selected.

Stagonospora meliloti (Lasch) Petr. Susceptibility to spotting of leaves seems common, if not general. Stem blight is found more abundantly in some slender-stem lines than in common coarse-stem varieties. However, selected lines of coarse, white sweetclover have shown almost uniform and severe infection.—F. R. JONES, J. L. ALLISON and W. K. SMITH, Division of Forage Crops and Diseases, Bureau of Plant Industry and Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

REPORT OF THE 1941 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1941 Annual Meeting of the Southern Division of The American Phytopathological Society was held as a section at the Meeting of the Association of Southern Agricultural Workers February 5, 6, and 8, in Atlanta, Georgia. A joint session was held with the Agronomy Section on diseases of forage crops. About 60 plant pathologists were in attendance at the meeting, and approximately 30 papers were given. A short business session was held on the morning of February 6, when the following officers were elected:

President, A. A. Dunlap
Vice-President, Luther Shaw
Secretary-Treasurer, I. L. Forbes

Titles and abstracts of papers presented at the meeting follow.

LUTHER SHAW
Secretary-Treasurer

Correlation of Spore Load with Incidence of Infection in Red Rot of Sugar Cane. I. L. FORBES. Inoculation experiments with *Colletotrichum falcatum* to determine the relation of spore load to incidence of infection were made over a period of 4 years, 1937–1940. Inoculations were made on cut stalks mainly, although standing cane also was inoculated. Commencing with a heavy spore suspension, 3 to 5 dilutions were made of about 1–10, 1–100, 1–1000, and 1–10,000. In some tests the approximate number of spores used as inoculum in each dilution was determined. Both resistant and susceptible varieties were inoculated. Inoculations of susceptible or resistant varieties with heavy spore suspensions resulted in a general rot in the inoculated internode due to the great number of individual infections that, because of their close proximity, fused, even in resistant varieties where individual lesions are limited in size. In internodes other than the one inoculated, lesions were progressively fewer the farther removed from the point of inoculation. The more dilute the spore suspension used as inoculum, the lower the number of lesions produced. In varieties highly resistant to lateral spread of the red rot, the spore load thus determined the severity of rot in an infected stalk. In more susceptible varieties, where individual lesions become much larger than in resistant varieties, obviously less inoculum—a lower spore load—would result in as much damage. Preliminary evidence is at hand indicating that the loss in virulence of the red rot fungus, when kept for some time in artificial culture, is due to a marked decrease, or perhaps to an actual loss in some cases of sporulation in such cultures.

Snap Bean Tissues Affected with Black Root. WILBERT A. JENKINS. The peculiar external manifestation of such symptoms as streaking and wilting of certain plant parts has been shown to be correlated histologically with differential necrosis of the phloem, cambium, and innermost xylem in the root, hypocotyl, stem, and pod. Necrotic symptoms in the phloem varied from slight cytoplasmic disorganization in cells recently infected through stages of more or less complete destruction of the cytoplasm and nucleus. In the terminal phases the cell contents resembled gum and the cellulose walls were covered by deposits of suberin. Callose pads also were noted in sieve tubes showing early symptoms of disease. Similar symptoms were exhibited by the cambium, except that symptoms in the meristematic zone closely resembled those of the respective contiguent vascular systems. In the xylem vessels very little evidence of plugging was observed except in the scalariform perforations. The xylem parenchyma about the youngest vessels contiguous to the cambium was severely necrosed, and necrosis occasionally extended into the ray parenchyma. Necrosis of the phloem was observed to extend throughout the vascular system of the pod and through the bundles of the funiculi into the seed coat at the chalaza. No evidence of embryonic infection was seen.

Some Leaf Spots and Berry Rots of Muscadine Grapes in Georgia. WILBERT A. JENKINS. Black rot on Muscadines is predominantly a foliage disease, although at times it may cause other symptoms. Early symptoms consist of water-soaked areas, which later become reddish-brown. The causal organism differs significantly in its life history, morphology, and pathogenicity from *Guignardia bidwelii* on bunch grapes and will be so described elsewhere. It is suggested that this disease be called muscadine black rot. A foliage disease caused by *Cercospora brachypus* is perhaps as prevalent in Georgia as muscadine black rot. Symptoms begin as small, angular chlorotic areas which enlarge and become necrotic in the center with a narrow halo about the angular lesion. The causal organism possesses a Mycosphaerella perfect stage which will be described elsewhere as a new species. It is suggested that this disease be known as Angular leaf spot of muscadines. Three destructive berry rots apparently are caused by new species of *Macrophoma*, *Melanconium*, and the above-mentioned *Guignardia*, respectively. Macrophoma rot is a soft rot that rarely mummifies the berry, Melanconium (bitter rot) mummifies the berry, while muscadine black rot rarely rots the entire berry, except in conjunction with Macrophoma rot or bitter rot.

Control of Nursery Blight of Pecan Seedlings by Spraying with Low-lime Bordeaux Mixture. JOHN R. LARGE and JOHN R. COLE. In seasons of excessive rainfall pecan nursery blight becomes one of the most important limiting factors in the production of budded pecan trees, especially in the Southeastern States. The fungus causing nursery blight, the *Sphaceloma* stage of (*Elsinoe randii*) invades both young and old leaflets, and infections result in small reddish lesions that develop on both surfaces of the leaves. Later in the season the spots on the upper surface of the leaves turn ash-gray. Single lesions usually are about ⅛ in. in diameter. These spots, however, may unite and form a continuous lesion along each side of a vein. Late-season infections are most numerous along the midribs and larger veins. To control nursery blight in the Southeastern States, the first application of 4–1–100 Bordeaux mixture should be made April 5 to 15, and be followed by 3 applications of 6–2–100 Bordeaux mixture. These applications are made at monthly intervals, with the last one about July 10. By following this spray program the nursery blight was controlled in 1939 and 1940 with resulting increased growth and vigor of the seedlings and the production of nearly 4 times as many successfully budded trees from the sprayed as from the nonsprayed trees.

Mode of Action of Certain Cotton-seed Treatment Materials. S. G. LEHMAN. When preparations containing certain organomercury compounds are used for dusting cotton seed prior to planting, considerable improvement in seedling emergence and survival results because of control of certain organisms commonly carried on the seed. The benefit is not confined to seeds that actually receive particles of the organomercury compounds in the dusting operation, but other seeds in the treated lot are benefited by reason of the action of the vapors that arise from these compounds. Vapors arising from ethyl mercury chloride, ethyl mercury phosphate, ethyl mercury borate, and ethyl mercury iodide are active in killing seed-borne spores and mycelium of *Colletotrichum gossypium, Fusarium moniliforme, Rhizopus nigricans,* and species of *Aspergillus.* Exposure of air-dry conidia of *Glomerella gossypii* on filter paper to vapors of these materials for periods as short as 1 to 4 hours resulted in failure of the conidia to grow when placed on agar. The vapors from the preparations containing the chloride and the phosphate appear to be somewhat more effective than those from the borate and iodide. When cotton seeds are exposed to these chemicals the vapors condense on the seed coat or lint that may be carrying the fungi, and become fungicidally active when the fungi become moist.

Downy Mildew on Oats in Mississippi. L. E. MILES. Downy mildew, *Sclerospora macrospora,* was discovered on oats near Indianola and Inverness, Sunflower County, Mississippi, in late spring, 1939. Brief surveys in 1940 showed it present in Sunflower, Sharkey, Leflore, Washington, and Oktibbeha Counties. The fungus was found on a few volunteer wheat plants in an infested field near Leland in Washington County. This is the first report of downy mildew on oats in the United States and the first report of its occurrence on wheat in Mississippi.

Affected plants tend to be stiff and leaves are more fleshy and upright than normal. Viable seed is not produced on infected plants. Fruiting panicles are frequently much distorted. Rachis and rachilla become curled and twisted. Spikelets, instead of bearing 2 seeds in close contact in the same glumes, produce a single seed-like structure in the glumes, which envelops the rachilla.

Oospores only are present. Oospores from wheat, oats, barley, corn, rice, and a number of wild grasses from various parts of the United States, Italy, Japan, and Australia have been compared with respect to diameter. These have agreed rather consistently. However, all specimens from Japan and one on *Avena fatua* from New South Wales have averaged much smaller.

Effect of Storage of Treated Cotton Seed in Closely Woven Cotton Bags. L. E. MILES. Cotton seed from the 1938 crop was mechanically delinted and treated with New Improved Ceresan in June, 1939. A part was stored in a 100-lb. bag and another in a closely woven cotton bag. Lots of the 1939 crop were (1) treated and stored similarly, and (2) stored in a burlap bag, nontreated. Germination tests November 1, 1939, showed 96.0 per cent from the 1938 crop lot treated and stored in burlap, and 98.0 per cent from the 1939 crop lot; both germinated 94.5 per cent on April 1; lots stored in cotton bags showed 95.5 per cent for the 1938 crop and 95.0 per cent for the 1939 crop on November 1, and both 92.0 per cent on April 1. Nontreated 1939 seed stored in burlap germinated

94.0 per cent on November 1, and 89.0 on April 1. In the field in 1940, the 1938 seed treated and stored in burlap germinated 34.7 per cent; and treated and stored in cotton, 35.05 per cent. Of the 1939 crop lot, the nontreated burlap lot germinated 21.45 per cent; the treated burlap lot, 39.4 per cent; and the treated lot stored in cotton, 40.5 per cent. New Improved Ceresan increased emergence over no treatment by 86.36 per cent, and yield by 22.71 per cent. These results indicate no injurious effects from storing treated cotton seed in cotton bags 17 months, and significant increases in both emergence and yield as a result of Ceresan treatment.

Fungi Associated with Diseased Cotton Seedlings and Bolls in 1938, 1939, and 1940. PAUL R. MILLER AND RICHARD WEINDLING. Diseased cotton seedlings and bolls collected in 14 States in 1938, 1939, and 1940 were cultured to determine the relative frequency and distribution of fungi associated with damping-off and boll-rots. Samples of diseased seedlings were taken from each of 1015 fields, of which 84 per cent showed *Glomerella gossypii;* 81 per cent, *Fusarium moniliforme;* 14 per cent, *Rhizoctonia solani;* 51 per cent, *Fusarium* spp. and 4 per cent, *Diplodia gossypina. Alternaria* spp., *Penicillium* spp., *Aspergillus* spp., *Pythium* spp., *Rhizoctonia bataticola* and *Sclerotium rolfsii* were isolated less frequently. Of the 744 boll-rot samples 73 per cent showed *Glomerella gossypii;* 70 per cent, *Fusarium moniliforme;* 87 per cent, *Alternaria* spp.; 65 per cent, *Fusarium* spp. and 15 per cent, *Diplodia gossypina.* Species of *Aspergillus, Rhizopus, Penicillium,* and a few unidentified organisms were isolated less frequently. *Glomerella gossypii* was not found on either seedlings or bolls west of eastern Texas and Oklahoma. Comparison of weather records with disease occurrence indicates that the distribution of anthracnose is correlated with summer humidity. Low humidity probably prevents survival of the causative organism during summer. The distribution of the other organisms was general throughout the area surveyed and there was little variation in frequency of occurrence from year to year.

Cotton-seed Treatment Tests in Louisiana in 1940. D. C. NEAL. In tests C-1 and C-2, consisting of 14 new materials, modifications, and combinations of certain chemicals previously used, and planted at Baton Rouge, Louisiana, on April 23, the best emergence values in C-1 were obtained in the order named from Ceresan 5 per cent, ethyl-Hg-borate, Ceresan 2 per cent, U. S. Rubber No. 120, and non-dusty 5 per cent Ceresan with tracer-dye modification. The increases in emergence with these chemicals over nontreated seed were highly significant. Sanoseed gave no improvement in emergence. In test C-2 the 4 materials giving highly significant increases were U. S. Rubber No. 98, Celatox, cyanamid, and copper mercury.

In test C-1, also, planted at St. Joseph, Louisiana, on May 6, and including in addition *mechanically delinted seed,* the mean emergence increases for all treatments were highly significant.

Also, a highly significant difference in favor of mechanically delinted over fuzzy seed was obtained at this locality. The effects of treatment on yield were not recorded because of prolonged unfavorable weather following planting.

1940 Regional Cotton-wilt Studies: Phosphate-variety Tests in Louisiana. D. C. NEAL. This test consisting of 7 varieties of cotton and 2 phosphate levels in the fertilizer (6–6–6 and 6–12–6) was planted at Baton Rouge and St. Joseph, Louisiana. The total wilt infection at final count at Baton Rouge ranged from zero per cent for Delfos 425 and Wanamaker S. & C. to 61 per cent for Half and Half, with the amount in the 4 other varieties ranging from 1 to 4 per cent, and falling in the same resistant group with Delfos 425, etc. Increasing the phosphate content of the fertilizer had no significant effect on the prevalence of wilt or in increasing yields. The 3 varieties giving the highest yield of seed cotton were Dixie Triumph 06, D. & P. L. 12, and Miller 610.

At St. Joseph, the rank in wilt resistance was nearly the same as at Baton Rouge, the results showing Half and Half the most susceptible (38 per cent wilt), Miller 610 next (12 per cent wilt), and the other 5 varieties falling into the most resistant class (infection range zero per cent for Wanamaker S. & C. to 4 per cent for D. & P. L. 12). Increasing the phosphorous in the fertilizer again did not increase the incident of the disease. The 5 varieties giving highest increases of seed cotton and falling within the same significant subgroup were D. & P. L. 12, Dixie-Triumph 06, Coker's 4 in 1, Miller 610, and Delfos 425. The yields of all varieties were highly significant over Half and Half, and the yield of D. & P. L. 12 (1939 pounds per acre) also was significant at the 1 per cent point over Wanamaker S. & C.

The Effect of Various Fungicidal Dusts upon Cantaloupes in Seasons Characterized by a Low Incidence of Downy Mildew. C. J. NUSBAUM. In 1939 and 1940 downy mildew remained at a low level in cantaloupes in the vicinity of Blackville, South Carolina, thus affording opportunity to studying the effect of several dust fungicides on the crop itself in the comparative absence of disease. Various fixed coppers, copper-lime, sulphur, and 2 organic fungicides were used. Attention also was given to the performance of various diluents and supplementary materials. In 1939 data were obtained upon defoliation and yield. In 1940 additional records were taken on age at maturity, weight and total solids in the juice of each fruit harvested from one plot of each treatment. In 1939 several fixed coppers and an organic material gave higher yields than the nontreated checks, but only a few of the increases exceeded the least significant difference. Some of the fixed copper and the copper-lime treated plots ranged slightly lower in yield than the checks. Sulphur mixtures reduced yields drastically. In 1940 yields from most of the fixed copper plots ranged higher than the checks, whereas the yield from copper-lime, yellow copper oxide, and Organic No. 244 plots were lower. Data from individual fruits indicated that none of the treatments delayed maturity or affected the weight and quality of marketable fruits significantly.

The Role of Hot Water Seed Treatment in the Control of Cercospora Blight of Benne. C. J. NUSBAUM. Recent outbreaks of blight (*Cercospora sesami*) on benne (*Sesamum indicum*) in the coastal region of South Carolina were brought to the writer's attention in 1939. Serious losses were reported from some hunting preserves where this crop was grown extensively as food for doves. The importance of seed-borne inoculum was determined when various seed samples from South Carolina, Georgia, and Florida showed high percentages of contamination with some lots showing as much as 16 per cent internally infected seed. Seed-treatment tests with hot water at temperatures ranging from 118 to 131° F. at 10-minute intervals up to 1 hour showed that a 30-minute treatment at 128° F. virtually eliminated seed-borne inoculum without injuring the seed appreciably. Based on a 30-minute treatment, the critical killing temperature for the internally borne pathogen was between 125 and 128° F. and the threshold of seed injury was between 128 and 131° F. Surface-borne inoculum was eliminated by treatment at 118° F. for 30 minutes. Heavily contaminated seed, held over 1 year in storage became free of surface-borne inoculum but internal infection remained. The practical value of hot-water disinfection was demonstrated in 1939 and 1940 on large-scale plantings at Ridgeland, South Carolina.

Copper-sulphur Dusts and Copper Sprays Give Good Control of Peanut Leaf-spot Diseases. LUTHER SHAW and T. T. HEBERT. Results of three years of experimental tests show that copper-sulphur dusts, ranging from 5 to 20 parts of copper and 95 to 80 parts of sulphur, and certain copper sprays, including Bordeaux mixture and cuprous oxide spray, have consistently given practical control of peanut leaf spots and economical increases in the yields of both nuts and hay. The most effective and practical schedule to follow in the application of these fungicides to peanuts is to make 4 applications at 2-week intervals beginning between July 1 and 10. Such treatments have produced increases in yields averaging about 500 lb. of peanuts and 800 lb. of hay per acre, at a cost of about $5.00 per acre, including both materials and labor. The approximate value of the increased yields of peanuts and hay per acre is $15.00. Studies on the efficiencies of different fungicidal materials in the control of peanut leaf spots have not progressed to the point where definite efficiency values can be given to the various materials under consideration.

Pathogenicity Tests of Different Isolates of Fusarium vasinfectum in 1940. C. D. SHERBAKOFF. In the summer of 1940 a test was carried in sand cultures in greenhouse with 5 isolates of *Fusarium vasinfectum* used for inoculation of 4 different varieties of cotton: Miller, Rowden, Delfos 719, and Cook 85. The results show a considerable difference in the virulence of the different strains, from 14.5 per cent to 43.6 per cent average, for the varieties of cotton used. A definite difference in the relative susceptibility of the different varieties is also indicated, Rowden being the more and Delfos 719 the least susceptible. The relative susceptibility is, however, about the same to all of the isolates used, thus indicating no specialization of any of the isolates in regard to their pathogenicity to the varieties of cotton.

Regional Studies of Fusarium Wilt of Cotton in Tennessee, in 1940. C. D. SHERBAKOFF. In 1940 the Regional Wilt Studies were carried again in cooperation with the U. S. Department of Agriculture, on the same general basis as the preceding 2 years, except that only 5 varieties of cotton were used: Miller 610, Coker's 4 in 1, Delfos 965–425, Early Wilt, and Dixie Triumph 06. The season's weather greatly reduced the wilt incidence and damage, strikingly so at Tiptonville. Of the varieties tested, Miller 610 was the highest in yield at Martin and nearly at the bottom at Tiptonville, while Coker's 4-in-1 gave the highest yield at Tiptonville and was near the bottom at Martin.

Regional Cotton-variety-wilt-phosphorus Study. A. L. SMITH. Cooperative investigations were conducted by members of the Fusarium Wilt Committee in seven States at 14 locations to study the effect of increased phosphorus on yield and fusarium wilt of 6 varieties of cotton. The fertilizer treatments were 600 lb. per acre of a 6–6–6 and 6–12–6. The varieties were Coker 4-in-1 strain 4, Early Wilt, Delfos 425, Dixie Triumph 06–366, Miller 610, and Half and Half.

The mean wilt in tests conducted in North Carolina, South Carolina, and Georgia was 10.9 per cent at the 6 per cent level of phosphorus and 11.9 per cent at the 12 per cent level of phosphorus. In central southern States the mean wilt was 10.4 per cent and 10.1 per cent at the same respective phosphorus levels. Yields were affectd very little by the increased phosphorus.

Coker 4-in-1 strain 4 and Early Wilt, two varieties highly wilt and root-knot-resistant, gave highest yields in the southeastern States in the order named. Dixie Triumph 06–366 and Miller 610, a wilt-resistant and a semi-resistant variety, and both root-knot-susceptible, were the highest-yielding varieties in the central-southern locations. There is strong circumstantial evidence to indicate the importance of both root-knot and wilt resistance in varieties to be planted in coastal-plains soils of the Southeast.

Nematode Distribution in the 1940 Regional Cotton-wilt Plots. A. L. SMITH AND A. L. TAYLOR. Cooperative investigations were conducted by members of the Fusarium Wilt Committee at 15 locations in 8 States. The root-knot-nematode infestation was determined by examining 3 cotton plants from each of 8 replicate 100-ft. plots. Infestation varied from 100 per cent at Scott, Mississippi, to none at Baton Rouge, Louisiana. The meadow nematode infestation was similarly determined from interplanted Crowder peas dug 3 weeks after planting. Infestation varied from zero to 70 per cent. A pronounced infestation of the reniform nematode (*Rotylenchulus reniformis*) occurred on roots of both cotton and cowpeas from Baton Rouge. In general there was a negative relationship between the root knot infestation and mean varietal-wilt reaction. The relative differences in the percentages of wilt in Half and Half (wilt-susceptible variety, intermediate in root-knot resistance) and Miller 610 (semi-resistant to wilt and highly root-knot-susceptible) is attributed to the variation in wilt and root-knot-nematode infestations at different locations. The wilt reaction of Half and Half more nearly reflected the prevalence of the wilt organism in the soil and was affected less by the prevalence of root knot. The wilt infection in Miller 610 was greatly reduced in comparison with Half and Half at two locations in one of which root knot was not present. In another the root-knot infestation was only 2 per cent. These results are in agreement with previous regional studies in which relative differences in susceptibility to wilt at different locations were attributed to varying nematode infestations and differences in varietal reaction to a combination of wilt and nematodes.

Mineral-deficiency Symptoms in Cotton in the Greenhouse. W. H. THARP and C. H. WADLEIGH. Kodachrome slides were shown of several stages of development of deficiencies of K, P, Mg, Ca, S, Mn, B, and Fe on Rowden 41–A cotton grown in aerated water culture in the greenhouse. The pattern of most of the deficiency symptoms was much the same as that reported for field conditions, but there was a failure of the red color to develop, even on severely affected leaves, in magnesium and potassium deficient solutions. The exclusion of ultra violet radiation by the glass is suggested as the main cause of this lack of red coloration.

A New Leaf Spot of Fig. E. C. TIMS. In August, 1939, an apparently undescribed leaf spot of fig, Celeste variety, was observed on a single tree near Houma, Louisiana. In late summer, 1940, it was again observed in several plantings in the same vicinity. Infection apparently does not appear until after most of the figs have ripened. For this reason it probably will cause little damage. The spots are light brown and usually have a darker brown border. A number of irregular concentric rings usually are found on the upper surface of the spots, and numerous, small, white, cushion-like fruiting structures of the fungus are conspicuous on the lower surface. The centers of the larger spots, 4–5 cm., often fall out, giving the leaves a very ragged appearance. Repeated isolations of the organism, tentatively identified as *Cephalosporium acremonium* (Corda), have been made and its pathogenicity established. Under favorable conditions of warm, moist weather, the spots enlarge rapidly on inoculated leaves. Leaf infection appears to take place in the absence of wounds.

Fusarium Hypocotyl Rot of Lupines. J. L. WEIMER. A destructive rot of the hypocotyl of *Lupinus luteus* L. was found at Quincy, Florida, in the spring of 1939. Diseased plants have since been received from Gainesville, Florida, and have been collected at Tifton and Americus, Georgia, and Auburn, Alabama. *L. angustifolius* and *L. albus* also are attacked, although *L. luteus* and *L. albus* apparently are more susceptible than is *L. angustifolius*. Older plants are not immune; young ones often are killed by severed hypocotyls. The hypocotyl-rot lesions may be mahogany red, chestnut, chocolate, or almost black, varying with the moisture and host species. Frequently, the lesion surface exhibits one or more usually longitudinal fissures. The necrotic tissue sinks, forming a typical canker. Often the hypocotyl just above and below the canker is somewhat enlarged. In the final stages of the disease, tops of affected plants are dwarfed, leaves turn yellow, wilt, and the plant dies. The disease is caused by a species of *Fusarium* belonging to the *Martiella* group, but the species has not yet been definitely determined. Isolates from several sources readily reproduce the disease when the inoculum is placed against the underground part of the hypocotyl without wounding it. An isolate of the *Fusarium* from cotyledon lesions reproduced the disease in inoculated plants, indicating that it is seed-borne. The disease, however, has been so widespread in some plats that it seems probable the fungus is more or less common in the soil. (Cooperative investigation between U. S. Department of Agriculture and Georgia Agricultural Experiment Station.)

SOME HYPHOMYCETES PARASITIC ON FREE-LIVING TERRICOLOUS NEMATODES

CHARLES DRECHSLER

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Agar plate cultures, prepared in the isolation of parasitic oomycetes from discolored roots or other decaying plant materials, often permit abundant multiplication of free-living nematodes, which then are frequently destroyed in large numbers by various predaceous and parasitic fungi. As the parasitic manner of attack, wherein infection comes about from germination of affixed or ingested spores, is shared widely among fungi, it is not surprising that the parasitic forms destructive to eelworms are rather diverse in their taxonomic relationships. Thus, while the 3 parasites that I described earlier (9, 10) under the binomials *Euryancale sacciospora, Haptoglossa heterospora,* and *Meristacrum asterospermum,* all belong in the Phycomycetes, they are referable to 3 different groups within that class. Ten additional species, similarly parasitic, but, from their septate vegetative mycelium, obviously belonging with the higher rather than with the lower fungi, are described herein. Important or conspicuous differences in the morphology of their conidial apparatus divide these species into 4 categories, only one of which is represented by more than a single genus.

TWO FUNGI WITH CLAMP CONNECTIONS IN THEIR CONIDIIFEROUS HYPHAE

In maize-meal-agar plate cultures, planted with leaf mold originating from wooded tracts in and near Washington, D. C., nematodes of the genus *Bunonema* have often been found undergoing destruction by a fungus more robust than *Euryancale sacciospora,* yet somewhat similar to that curious zoopagaceous parasite in the ranginess of its reproductive filaments. To initiate infection the elongated conidium of the fungus attaches itself usually to the anterior portion of an eelworm by means of a short adhesive process arising from the narrower of its two ends (Fig. 1, A, a). This process perforates the integument and then continues growth in extending a system of assimilative hyphae longitudinally through the animal. When the fleshy contents have been largely appropriated the assimilative mycelium gives rise to several branches that erupt from the integument and grow over the surface of the substratum. Until they attain some length the external hyphae remain continuous (Fig. 1, A, b, c, d). Indeed, ordinary crosswalls are never laid down in these filaments, for deposition of septa takes place here only in the formation of clamp connections, the latter process, again, in most instances being closely associated with spore production (Fig. 1, B–N).

The hyaline, somewhat fusiform conidia (Fig. 1, O, a–o), which serve in the infection of host animals, are formed terminally on erect, tapering, aerial sterigmata (Fig. 1, B, c–g, i–l, q, s–w) arising from the prostrate hyphae at intervals varying usually between 30 μ and 50 μ. After 1

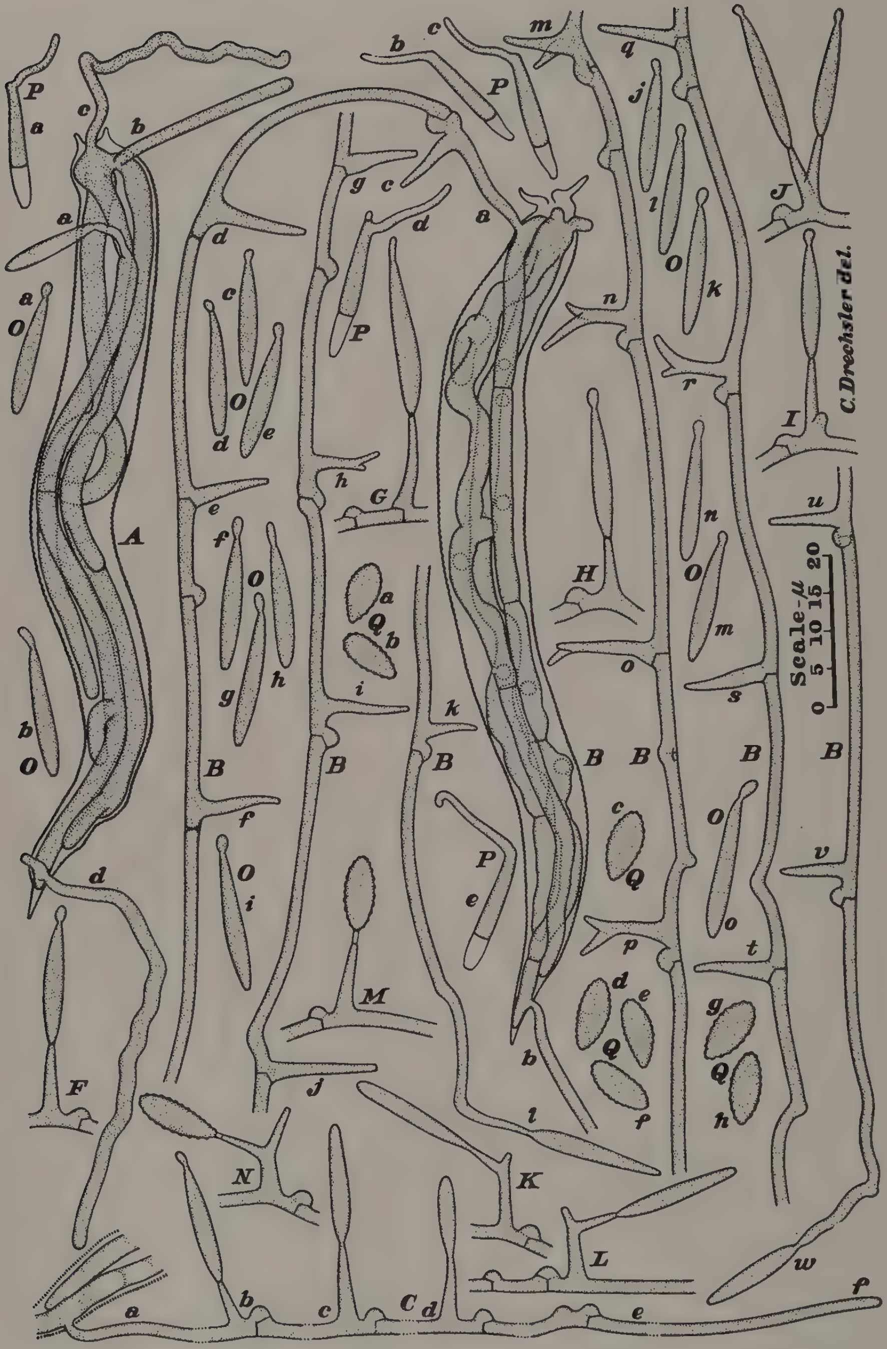
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FIG. 1. *Nematoctonus tylosporus.* For legend see opposite page.

conidium has been cut off the sterigma often grows out laterally, thereby giving rise to a spur on which a second conidium is produced (Fig. 1, B, h, m–p, r; J–L). Occasionally the process is repeated, with resultant formation of a third spore. Although the sterigma is sometimes borne directly on a clamp connection (Fig. 1, B, e, g, j, o, q, s, t; G), it more frequently arises a short distance backward from a clamp, that is, nearer the origin of the parent filament (Fig. 1, B, c, d, f, h, i, k, m, n, p, r, u, v; C, b, c, d). Here and there supernumerary clamp connections may be found that have no close positional relationship to any sterigma. The sequence in development of sterigmata and of the clamps associated with them would seem subject to irregularity, since some growing conidiiferous filaments are found terminating in an ascending sterigmatic part (Fig. 1, B, l, w), while others are found bearing 1 or 2 clamps beyond the most distal of their sterigmata (Fig. 1, C). The interrelationships here appear rather closely similar to the interrelationships in the clamp-bearing conidiiferous hyphae of *Corticium incrustans* Höhn. and Litsch. (= *C. roseopallens* Burt), as illustrated first by Lyman (22) and, more recently, with cytological detail by Nobles (25). Somewhat comparable arrangement of parts is evident also in one of Vandendries' figures (40, Fig. 7) showing a diploid conidiiferous branch of *Pleurotus pinsitus* Fr.

Before a conidium attains full maturity, and while it is still attached to the sterigma, its elongated fusiod shape is modified through the production of a small, mostly knob-like, apical excrescence (Fig. I, F–J; O, a–o). Apparently, this protuberance is more or less adhesive, and thus may well be of service for a time in helping to fasten the spore to a prospective nematode host. However, after the conidium has fallen off onto the substratum, the distal knob usually suffers obliteration, owing to the production of an erect or ascending hyphal outgrowth from the apex of the spore (Fig. 1, P, a–e). This outgrowth, which usually arises somewhat obliquely with respect to the conidial axis, would seem strongly adhesive, and undoubtedly is efficient in attaching the spore to a suitable eelworm. The material required for putting forth the hyphal process is obtained through evacuation of a basal

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FIG. 1. *Nematoctonus tylosporus*, drawn to a uniform scale with the aid of a camera lucida; × 1000 throughout. A. Specimen of *Bunonema* sp. permeated with assimilative hyphae of the fungus: a, conidium by which the animal was infected; b, c, d, young conidiophorous filaments. B. Specimen of *Bunomena* sp. permeated with assimilative hyphae from which 2 conidiophorus filaments, a and b, are being extended; for lack of space these filaments are shown in parts, the proper continuity of which is made evident in that the sterigmata arising from filament a are designated in acropetal order by the alphabetical sequence c to l, while those arising from the filament b are designated in acropetal order by the alphabetical sequence m to w. C. Six portions of a growing conidiophorous filament designated in acropetal order by the letters a to f; showing development of conidia on their sterigmata, and positional relationship of sterigmata to clamp connections. F–I. Portions of conidiophorous hyphae, each showing a fully developed conidium on a simple sterigma arising in close proximity to a clamp connection. J–L. Portions of conidiophorus hyphae, each showing a branched sterigma close to a clamp connection. M, N. Two sterigmata, each bearing a resting spore. O, a–o. Conidia as abjointed from their sterigmata, showing variations with respect to size, shape, and terminal modification. P, a–e. Detached conidia, each bearing an adhesive apical outgrowth. Q, a–h. Resting spores showing variations in shape and size.

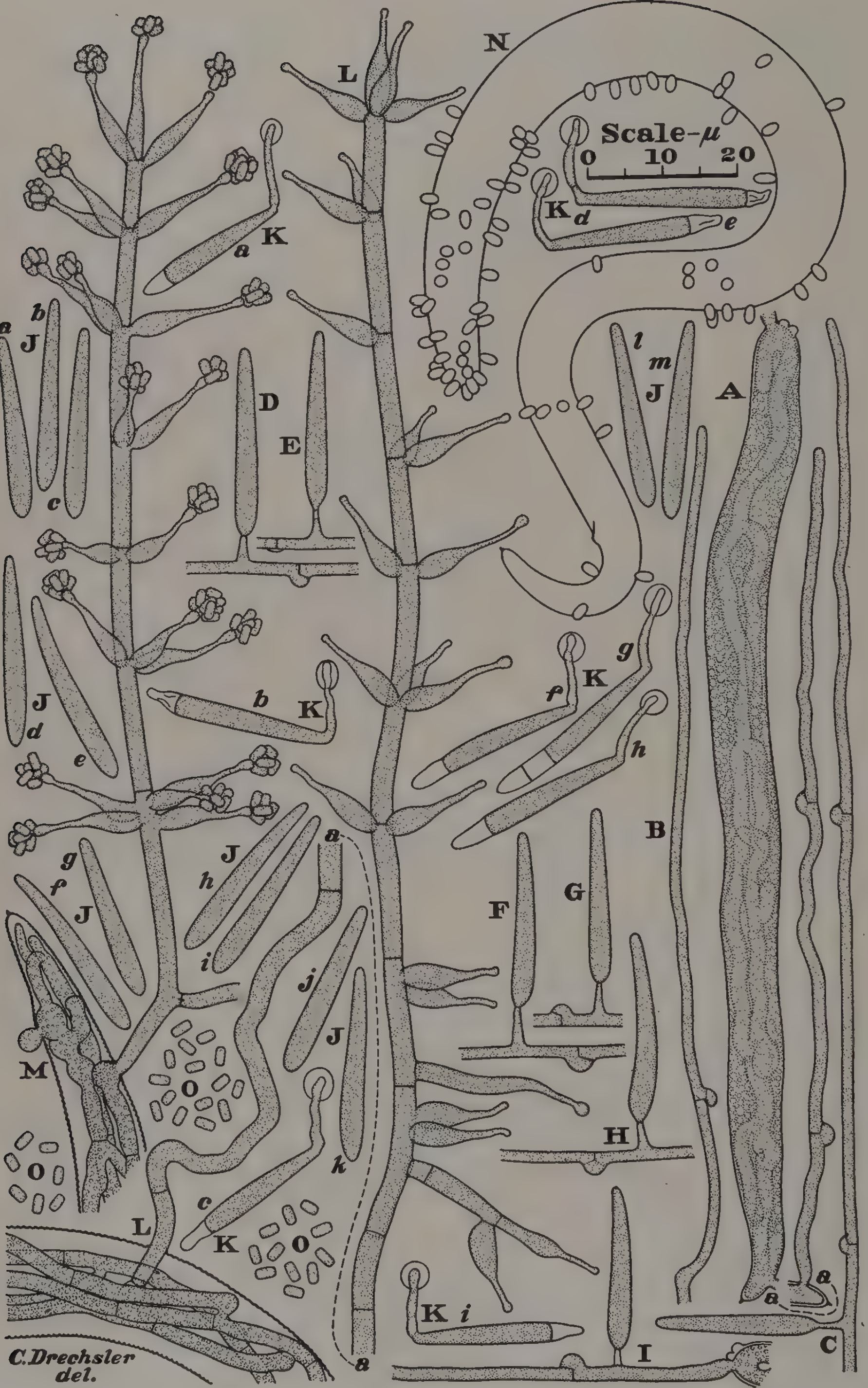
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FIG. 2. A–K. *Nematoctonus leiosporus.* L–O. *Acrostalagmus bactrosporus.* For legend see opposite page.

portion of the conidium. As the process, on attaining a length equivalent to $\frac{1}{2}$ or $\frac{2}{3}$ the length of the spore, makes no further growth, except in the infection of a nematode, its production must be considered rather different from the ordinary type of vegetative germination whereby saprophytic fungi initiate development of a new mycelium.

Occasionally, in place of the smooth fusoid conidia hitherto discussed, the sterigmata of the fungus bear shorter, wider, prolate ellipsoidal spores, of minutely verrucose sculpturing and faintly yellowish coloration (Fig. 1, M; N; Q, a–h). These spores have not so far been seen to germinate, or to bring about infection of nematodes. In the rather meager material affording their production it was not apparent that they are formed in asymmetrical relationship to the sterigmata, or that they are shot away by a drop-secretion mechanism after the manner of basidiospores in the Hymenomycetes and Uredineae (3). The accessory reproductive bodies may perhaps best be considered as resting conidia adapted for survival over unfavorable periods, and, therefore, corresponding more or less closely to the asexual spores of terminal origin designated by Mason (24) as aleuriospores.

A closely related fungus was found destroying eelworms in an old maize-meal-agar plate culture to which had been added some pinches of leaf mold collected in deciduous woods near Butternut, Wis., in September, 1938. The animals attacked by it included representatives of the genera *Rhabditis* and *Wilsonema,* as well as specimens of *Plectus parvus* Bastian (Fig. 2, A). During the earlier stages of infection the branching system of assimilative hyphae extending lengthwise through the fleshy body are always badly obscured by the innumerable globules resulting from degeneration of musculature and organs. The assimilative hyphae soon give rise to a number of branches, which, after erupting from the integument of the host, extend over the surface of the substratum. On the external filament thus brought into being clamp connections are formed at intervals (Fig. 2, A, a; B) and erect aerial conidia (Fig. 2, C–I) are produced in successively more distal positions until the protoplasmic materials of the fungus have been used up. The sterigmata here are appreciably shorter than those of the related species

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FIG. 2. Drawn to a uniform scale with the aid of a camera lucida; × 1000 throughout.

Nematoctonus leiosporus: A. Specimen of *Plectus parvus* occupied by branching assimilative hyphae, which are extending 2 conidiophorous hyphae; one of these hyphae, a, with its 2 clamp connections is shown separately. B. Terminal portion of a growing conidiophorus filament, showing a clamp connection in process of development. C. Terminal portion of a growing conidiophors filament, with 2 clamp connections and a young conidium. D–I. Portions of growing conidiophorous filaments, each showing a fully developed conidium borne on a sterigma arising a short distance proximad from a clamp-connection. J, a–m. Conidia showing variations in size and shape. K, a–i. Conidia, which, after falling on agar medium, have each put forth an erect adhesive process whose tip is inclosed in a droplet of adhesive secretion.

Acrostalagmus bactrosporus: L. Median portion of an invaded specimen of *Plectus parvus,* showing origin of a well developed conidiophore from an assimilative hypha—from lack of space the conidiophore is drawn in 2 parts, which are connected by a broken line; and the conidiiferous branches are shown without any of the conidia abjointed by them. M. Origin of a branched conidiophore from the posterior portion of an infected specimen of *P. parvus;* showing spore clusters attached to the conidiiferous branches. N. Small specimen of *P. parvus* to which numerous conidia of the parasite are affixed. O. Conidia showing variations in size and shape.

from Washington, D. C., and regularly conclude their development with the production of a single conidium. Greater length and a tapering digitiform shape distinguish the conidia of the Wisconsin fungus (Fig. 2, J, a–m) from those of the eastern species. Further, the conidia of the parasite from Wisconsin show no apical modification as long as they are supported aloft on their sterigmata. However, very soon after falling on the substratum they invariably burgeon forth an erect hyphal process from the distal end. When this process has attained a length equal to about one-third the length of the spore, it stops growing and secretes at its tip a glistening droplet of hyaline adhesive material (Fig. 2, K, a–i). In its position, nearly 10 µ above the substratum, the adhesive droplet is advantageously placed to operate in fastening the conidium to any nematode that may come creeping along on the surface. Production of the adhesive process is accomplished here, just as in the related species, through withdrawal of protoplasmic contents from the proximal end of the spore. Additional germinative development takes place only in the successful invasion of a host animal. The fungus consequently would seem to lack all capacity for saprophytic growth.

The presence of numerous clamp connections in their conidiophorous hyphae marks the 2 parasites as belonging unmistakably among the Basidiomycetes. Now, the large membership of this class of fungi has been little implicated in parasitism on animals. According to recent investigations by Couch (5) the genus *Septobasidium* subsists on scale insects through a relationship, which, though involving parasitism, is interpretable as symbiosis when broadly considered. *Uredinella coccidiophaga,* described by Couch (4) as the type of a new genus intermediate in morphology between the rusts and *Septobasidium,* likewise subsists through parasitism on scale insects. MacMillan (23) observed earlier that when swarms of small flies visited fruiting bodies of *Polyporus applanatus* (Pers.) Wallr., an occasional insect would get its feet caught between the clefts, and, being unable to extricate itself, would soon die. Its body thereupon would promptly become enveloped by new mycelial growth, and its substance utilized in the formation of additional hymenial pores. Such incidental utilization of nourishing material, as Sartory (35) pointed out, can hardly be held to constitute parasitism. *Hirsutella entomophila* Pat., originally described (28) in 1892 as a member of the Clavariaceae occurring parasitically on a coleopteron from Ecuador, and accordingly compiled by Guéguin (12) and Sartory (35) as a basidiomycete parasitic on an animal host, has been shown by Speare (39) to be a hyphomycete, very probably achieving its sexual stage as a species of the ascomycetous genus *Cordiceps.* As Petch (30) later rather definitely determined a pleomorphic connection between another species of *Hirsutella* and *Cordiceps,* Patouillard's fungus can no longer be reckoned among the basidiomycetes. Again, Sartory's subsumption under the Protobasidiomycetes of *Trichoceridium ramosum* Poisson and *Dixidium dixae* Poisson, both occurring as intestinal parasites of dipterous larvae (33), would seem to be sustained only in some meager resemblance that the sporulating branches of *D. dixae* bear to rust mycelia and to the transversely septate basidia in the genus *Auricularia. D. dixae*, moreover, appears not to be pathogenic to its host. By contrast, the 2 clamp-bearing fungi reveal in their destructive parasitism on motile animals a biotic relationship exceptional among basidomycetes—so exceptional, indeed, that it must be considered a feature of primary taxonomic importance. Wherefore, instead of assigning the fungi separately to such unsuitable genera as *Cylindrophora* and *Acremonium*, I am placing them in a new mucedinaceous genus for which a name compounded of 2 words meaning "thread" and "murder," respectively, may not be inappropriate. The specific epithets applied to them, constructed in part of words meaning "knob" and "smooth" respectively, are intended to be descriptive of their conidia previous to disarticulation.

Nematoctonus, gen. nov.

Mycelium filiforme, hyalinum; hyphis assumentibus plus minusve ramosis, intra animalcula viventia evolutis; hyphis exteris plerumque sparsis, saepe repentibus, in modum Hymenomycetum septato-nodosis, hic illic ex apice sterigmatum erectorum simplicium vel parce ramosorum conidia hyalina etiam quandoque sporas dilute coloratas gignentibus.

Mycelium filamentous, hyaline; the assimilative hyphae, more or less branched, developing within minute living animals; the nonassimilating hyphae developing outside of animals, mostly sparse, often prostrate, provided with clamp connections, at intervals producing hyaline conidia, together sometimes with slightly colored spores, on simple or slightly branched ascending sterigmata.

Nematoctonus tylosporus, sp. nov.

Hyphae assumentes irregulariter ramosae, plerumque 2.5–3.5 μ crassae, intra vermiculum nematoideum viventem crescentes, post mortem animalis aliquot hyphas fertilis repentis emittentes; hyphis fertilibus septato-nodosis, vulgo .5–2 mm. longis, 1.5–2 μ crassis, conidia vel rarius sporas perdurantes ex sterigmatibus gerentibus; sterigmatibus erectis, vulgo 5–13 μ altis, basi 1.7–2.5 μ crassis, sursum attenuatis, apice .8 μ crassis, inter se 30–50 μ distantibus, primo simplicibus postae a latere ramulum brevem saepe emittentibus et sporam alteram proferentibus; conidiis continuis, incoloratis, fusoideis, plerumque 17–22 μ longis, 2.3–2.7 μ crassis, basi rotundatis vel rotundo-truncatis, apice tuberculo minuto praeditis, postea ibi hypham germinationis circa 10 μ longam 1 μ crassam oblique emittentibus; sporis perdurantibus continuis, flavidis, minute verrucosis, elongato-ellipsoideis vel obovoideis, 8–11 μ longis, 4–4.5 μ crassis.

Vermiculos nematoideos specierum Bunonematis necans habitat in humo silvestri prope Beltsville, Maryland, et in Washington, D. C., et in Arlington, Virginia.

Assimilative hyphae irregularly branched, mostly 2.5 to 3.5 μ wide, developing within living nematodes, after death of host animal extending several conidiophorous hyphae over the substratum; conidiophorous hyphae 1.5 to 2 μ wide, often attaining lengths of .5 to 2 mm., provided with clamp connections, producing conidia and more rarely resting spores on erect sterigmata; the sterigmata commonly 5 to 13 μ high, 1.7 to 2.5 μ wide at the base, tapering upward to a width of about .8 μ at the tip, spaced at intervals of 30 to 50 μ, at first simple but later often putting forth a short branch and giving rise to a second spore thereon; the conidia aseptate, colorless, spindle-shaped, mostly 17 to 22 μ long, 2.3 to 2.7 μ wide, rounded or somewhat truncate at the base, provided at the apex with a small knob, after disjunction often giving rise from the distal end to an incipient germ tube about 10 μ long and 1 μ wide; the resting spores aseptate, slightly yellowish, minutely warty, elongate ellipsoid or somewhat obovoid, 8 to 11 μ long and 4 to 4.5 μ wide.

Destroying nematodes referable to species of *Bunonema* it occurs in leaf mold near Beltsville, Md., in Washington, D. C., and in Arlington, Va.

Nematoctonus leiosporus, sp. nov.

Hyphae assumentes irregulariter ramosae, plerumque 2–3 μ crassae, intra corpora viventia vermiculorum nematoideorum crescentes, post mortem animalis aliquot hyphas fertilis repentis emittentes; hyphis fertilibus septato-nodosis, saepius .5–2 mm. longis, 1.5–2 μ crassis, conidia singulatim hic illic ex sterigmatibus gerentibus; sterigmatibus erectis, simplicibus, 2–5 μ altis, basi circa 1.5 μ crassis, sursum attenuatis, inter se 30–60 μ distantibus; conidiis continuis, incoloratis, digitiformibus, plerumque rectis, utrimque rotundatis, sursum attenuatis, 20–27 μ longis, 2.6–3.4 μ crassis, ex apice hypham glutinosam mox emittentibus; hypha glutinosa erecta, circa 10 μ longa, basi 1–1.5 μ crassa, apice guttulam 3–4 μ crassam ferente.

Vermiculos nematoideos (qui ex parte specierum Plecti et Rhabditis et Wilsonematis sunt) necans habitat in humo silvestri prope Butternut, Wisconsin.

Assimilative hyphae irregularly branched, mostly 2 to 3 μ wide, developing within living nematodes, after the death of a host animal extending several conidiophorous hyphae over the substratum; conidiophorous hyphae often .5 to 2 mm. long and 1.5 to 2 μ wide, provided with clamp connections at intervals of 30 to 60 μ, producing conidia singly on erect sterigmata; sterigmata simple, 2 to 5 μ high, about 1.5 μ wide at the base, tapering upward; conidia aseptate, colorless, digitiform, rounded at both ends, tapering gradually upward, mostly straight, at maturity measuring commonly 20 to 27 μ in length by 2.6 to 3.4 μ in width, after falling on the substratum giving rise at the apex to an erect adhesive process; this process often approximately 10 μ long, 1 to 1.5 μ wide at the base, and bearing at its tip a glutinous droplet 3 to 4 μ in diameter.

Destroying nematodes belonging in part to species of *Plectus, Rhabditis,* and *Wilsonema* it occurs in leaf mold near Butternut, Wis.

In framing the diagnosis of *Nematoctonus* consideration was given to another nematode-destroying fungus that, hitherto, I have seen only in material kindly sent to me from Hawaii by M. B. Linford. According to information supplied in a letter accompanying the material this fungus was observed to destroy larvae of *Heterodera marioni* (Cornu) Goodey, as well as specimens of *Aphelenchus avenae* Bastian and representatives of the genera *Rhabditis* and *Cephalobus,* in cultures prepared with washings from pineapple (*Ananas sativus* Schult.) roots collected at Wahaiawa, Oahu. Among the many fungus enemies of nematodes that came to light in similar cultures (19, 20) it was conspicuous through its production of clamp connections. Unlike the 2 clamp-bearing species from continental sources the Hawaiian fungus is predaceous in its mode of attack. To capture prey it utilizes erect hyphal processes bearing droplets of adhesive mucus at the tip. As the processes often contain from 1 to 3 cross walls and frequently measure 10 to 20 μ in length, they invite comparison more especially with the somewhat similar predaceous processes of *Dactylella gephyropaga* Drechsl. (8) during the earlier stages of development. The mycelial hyphae, which measure about 2.5 μ in width, bear at intervals on rather minute conical sterigmata strongly curved allantoid conidia approximately 20 μ long and 5 μ wide.

FOUR HYPHOMYCETES THAT FORM CONIDIA SUCCESSIVELY ON FLASK-SHAPED BRANCHES

In old agar plate cultures planted with decaying vegetable material specimens of *Plectus parvus* are often found undergoing destruction by a handsomely verticillate fungus. When the globulose, degenerating contents of an infected animal have been largely expropriated the vegetative stage of the parasite becomes discernible within as a septate branched mycelium filled with densely granular protoplasm. From this mycelium lateral branches are soon put forth, which, after breaking through the host integument, make their way to the surface of the substratum, and then extend into the air as erect or ascending conidiophores (Fig. 2, L, M). The several conidiophores originating from animals that happened to succumb deep under the substratum usually emerge at some distance from one another. In instances where an infected eelworm has succumbed on the surface, the several reproductive filaments for the most part grow directly into the air, so that their common origin from an individual host animal is immediately obvious.

The conidiophores, which, with respect to their main design, may be simple or sparingly branched, become divided by transverse septa into segments varying between 15 and 20 μ in length. Most of the segments give rise at the distal end to 2 or 3 flask-shaped conidiiferous branches, though some segments bear only 1 such branch, and others put forth as many as 5 (Fig. 2, L, M). Despite rather frequent irregularity of positional relationships the conidiiferous branches appear in predominantly verticillate arrangement. Each produces successively on its tapering sterigma about a dozen small conidia that remain attached in a cluster, evidently being held together by adhesive material (Fig. 2, M).

The presence of an adhesive substance is manifest likewise when the conidia become attached to host animals preparatory to infection. Larvae of *Plectus parvus* have often been found beset with dozens of spores (Fig. 2, N), every spore adhering by one of its ends. Although the foothold derived from such narrow contact looks very insecure, it is maintained in spite of the energetic squirming of the nematode.

The fungus appears eligible for inclusion in *Acrostalagmus,* since that genus would seem to be distinguished by verticillate arrangement of flask-shaped conidiiferous branches from each of which conidia are abjointed one after another to collect in a glutinous head. Various entomogenous species of *Acrostalagmus* have long been known, as, for example, *A. aphidum* Oudemans (26) and *A. coccidicola* Guéguin (12). Such entomogenous forms have presented some difficulties of classification owing to the circumstance that often they bear conidiiferous branches in part verticillately and in part singly; the single branches being approximately equivalent to the simple conidiophores of the genera *Cephalosporium* and *Hyalopus.* After considering representations by Guéguin (13) on the similarity of *Hyalopus* and *Acrostalagmus,* as well as representations by Parkin (27), Lindau (18, p. 100–101), and Buchanan (2) on the relationship of *Acrostalagmus* and *Cephalosporium,* Petch (31) concluded that it would be most expedient to place in *Cephalosporium* certain verticillate species occurring on scale insects,—a disposition he subsequently extended to similar verticillate forms on insect hosts generally. Nevertheless, in naming these fungi, Petch often mentions *Acrostalagmus* in parentheses; a new species growing on thrips, for example, being presented by him (32) as *Cephalosporium (Acrostalagmus) thripidum.*

As *Acrostalagmus,* erected in 1838, antedates *Cephalosporium* by a year, and as it was founded on a much more distinctive type of conidial apparatus, its abasement in favor of the latter genus seems somewhat inadvisable. I am, therefore, describing the verticillate parasite as a species of *Acrostalagmus* under an epithet intended to have reference to its bacillus-like conidium. The rather traditional interpretation of mucedinaceous genera followed herein should not result in any confusion of species; for, while the present fungus and the 3 related fungi next to be considered appear truly akin to the entomogenous forms subsumed by Petch under *Cephalosporium*, it is not evident from study of the relevant literature that any of the fungi hitherto found occurring as habitual parasites on eelworms are to be numbered among the parasites destructive to insects or spiders.

√ **Acrostalagmus bactrosporus**, sp. nov.

Mycelium nutritum hyalinum, ramosum, septatum, intra vermiculos nematoideos viventes evolutum, ex hyphis filiformibus 2–2.5 μ crassis compositum. Hyphae fertiles in aere 100–400 μ se porrigentes, sursum erectae vel ascendentes, hyalinae, plerumque 2–3.5 μ crassae, axe simplices vel parce ramosae, in cellulis saepe 15–20 μ longis constantes, quaque cellula 1–5 ramulos conidiferos magnam partem sursum ferente; ramulis conidiferis (phialis) plerumque binis vel ternis verticillatis, lageniformibus, saepius 10–15 μ longis, prope basim 2.5–3.5 μ crassis, quoque sursum in sterigma .5–1 μ crassum abeunte, et ex apice sterigmatis 5–15 conidia deinceps gerente; conidiis cohaerentibus, continuis, hyalinis, bacilliformibus, 2–3 μ longis, 1.3–1.6 μ crassis.

Plectum parvum necans habitat large in humo silvestri et alteris materiis plantarum putrescentibus prope Beltsville, Maryland, et in Arlington, Virginia.

Assimilative mycelium hyaline, branched, septate, growing within living nematodes, composed of filamentous hyphae 2 to 2.5 μ wide. Conidiophores extending 100 to 400 μ into the air, the aerial portion erect or ascending, mostly 2 to 3.5 μ wide, the axial filament simple or sparingly branched, and consisting of cells, often 15 to 20 μ long, each of which bears 1 to 5 conidiiferous branches mainly at its distal end; conidiiferous branches (phialides) mostly arranged in whorls of 2 or 3, lageniform, 2.5 to 3.5 μ wide near the base, each terminating in a sterigma .5 to 1 μ wide at the tip, whereon 5 to 15 conidia are borne successively; conidia aseptate, hyaline, bacilliform, 2 to 3 μ long, 1.3 to 1.6 μ wide, cohering in a head.

Destroying nematodes referable to *Plectus parvus*, it occurs abundantly in leaf mold and other decaying vegetable materials near Beltsville, Md., and in Arlington, Va.

In a few maize-meal-agar plate cultures to which had been added some friable detritus from disintegrating stems of the giant ragweed (*Ambrosia trifida* L.)—detritus collected in Arlington, Va., late in March 1938—eelworms of the same species often destroyed by *Acrostalagmus bactrosporus* were found being parasitized by an allied fungus. While this related species shows little individuality with respect to its vegetative development within the host animal, its conidial apparatus is readily distinguishable from that of other hyphomycetes subsisting on nematodes. The conidiophorus filaments, after erupting from the host integument and growing through the overlying material, extend often somewhat irregularly either by creeping along the surface of the substratum or by elongating into the air. On the prostrate or ascending portions of the filaments (Fig. 3, A–E) are borne conidiiferous branches, mostly of the familiar flask-shaped type, with a short sterigma distally prolonging the swollen basal part, though, occasionally, the sterigma may be borne laterally on the swollen part. The conidiiferous branches may arise singly or in pairs, or, again, in groups of 3, 4, 5, or 6. The more numerous groups, especially when attached to ascending portions of a conidiophorous filament, are often arranged verticillately. Because of

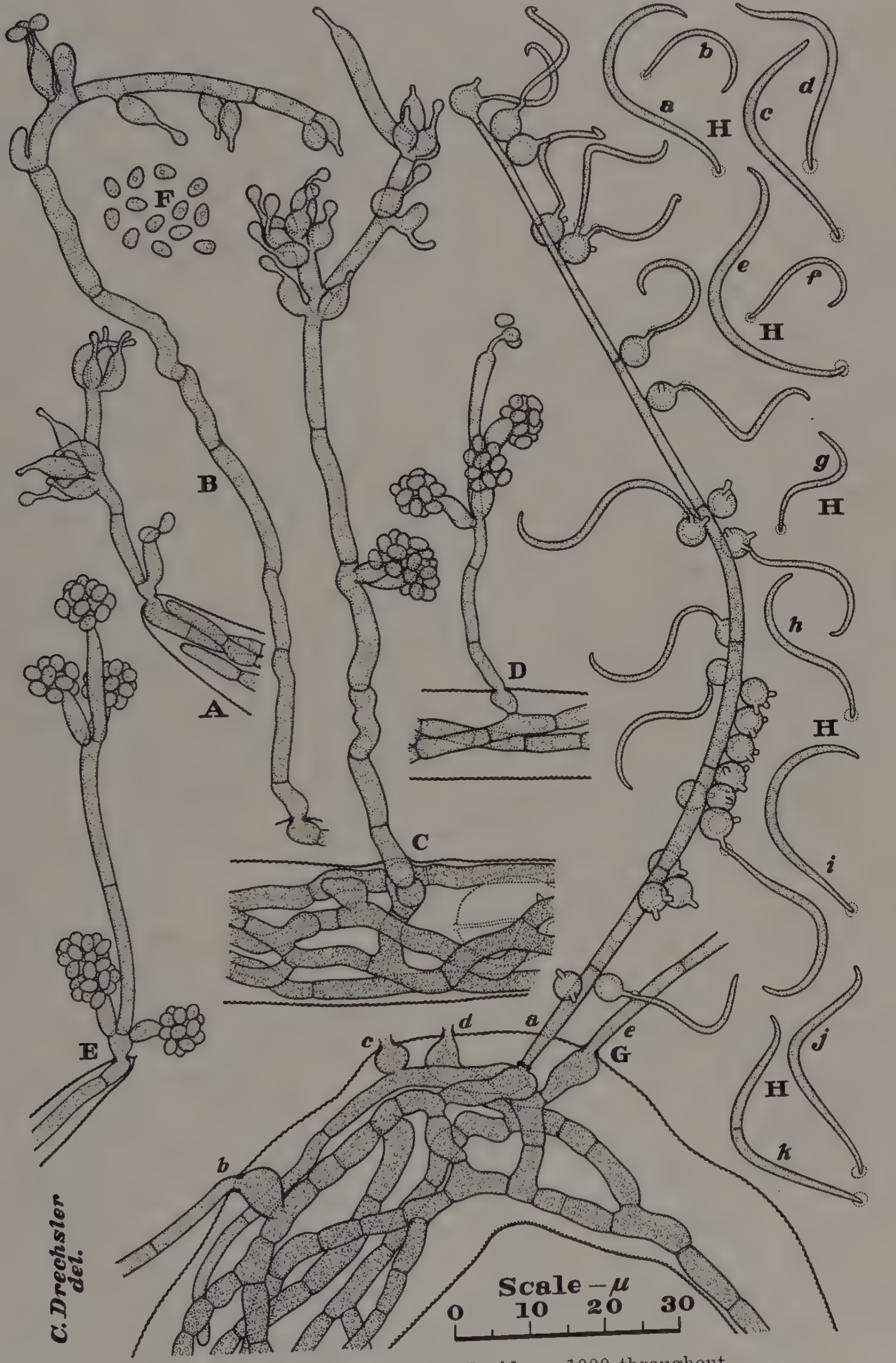

FIG. 3. Drawn with the aid of a camera lucida; × 1000 throughout.

Acrostalagmus obovatus: A, B. Conidiophorus hyphae shown with sterigmata mostly denuded. C. Conidiophorous hypha with all except 2 conidiiferous branches shown denuded of spores. D, E. Conidiophorous hyphae with spore clusters shown in position. F. Detached conidia.

Harposporium helicoides: G. Portion of infected specimen of *Rhabditis* from which 5 conidiophorous hyphae, a–e, have grown out; one of them, a, being shown entirely, though largely denuded of its conidia. H, a–k, Detached conidia.

this verticillation and the persistence of the successively abjointed conidia in coherent heads, the fungus seems most appropriately referable to the genus *Acrostalagmus*. Usually about 20 conidia are produced at the tip of a sterigma, but often several conidial clusters become confluent to form masses containing larger numbers of spores. A term meaning "like an inverted egg" approximately describes the shape of the conidium (Fig. 3, F) and is therefore used as the specific name to set the fungus apart from *A. bactrosporus*.

Acrostalagmus obovatus, sp. nov.

Mycelium nutritum hyalinum, ramosum, septatum, intra vermiculos nematoideos viventes evolutum, ex hyphis filiformibus 2–3 μ crassis compositum. Hyphae fertiles extra animal emortuum evolutae, repentes vel ascendentes, hyalinae, axe simplices vel parce ramosae, saepe 50–500 μ longae, plerumque 1.7–3 μ crassae, in cellulis saepius 15–20 μ longis constantes, nonnullis cellulis 1–6 ramulos conidiferos ferentibus; ramulis conidiferis (phialis) modo singulis vel binis modo 3–6 aggregatis vel verticillatis, vulgo lageniformibus, 6–10 μ longis, prope basin plerumque 2.5–3.5 μ crassis, quoque in breve sterigma .5 μ crassum abeunte et ex apice sterigmatis circa 20 conidia deinceps gerente; conidiis cohaerentibus, continuis, incoloratis, ellipsoides vel inversum ovoideis, circa 3 μ longis, 2 μ crassis.

Plectum parvum necans habitat in caulibus Ambrosiae trifidae putrescentibus in Arlington, Virginia.

Assimilative mycelium hyaline, branched, septate, growing within living nematodes and composed of filamentous hyphae 2 to 3 μ wide. Conidiophorous hyphae formed outside of the dead host animal, creeping or ascending, hyaline, the axial filament simple or sparingly branched, often 50 to 500 μ long, mostly 1.7 to 3 μ wide, consisting of cells often 15 to 20 μ long, some of which bear 1 to 6 conidiiferous branches; conidiiferous branches (phialides) sometimes borne singly or in pairs, and sometimes aggregated in numbers from 3 to 6 in whorls or more irregular groups, commonly flask-shaped, 6 to 10 μ long, mostly 2.5 to 3.5 μ wide near the base, each bearing terminally or more rarely laterally a short sterigma .5 μ wide and at the apex thereof producing in succession about 20 conidia; the conidia aseptate, colorless, ellipsoid or obovoid, about 3 μ long and 2 μ wide, cohering in a terminal head.

Destroying nematodes referable to *Plectus parvus* it occurs in decaying stems of *Ambrosia trifida* in Arlington, Va.

In several maize-meal-agar plate cultures, to which had been added small quantities of leaf mold collected near Haugen, Wis., in September, 1939, the same species of nematode attacked by the 2 verticillate hyphomycetes just described was found parasitized occasionally by a related fungus with rangier conidial apparatus. While this fungus attacked *Plectus parvus* only sparingly it parasitized *Acrobeloides bütschlii* (De Man) Thorne much more freely. Infected eelworms of either species often showed extensive separation of integument from musculature (Fig. 4, A). Usually, the closely septate hyphae of the assimilative mycelium was not clearly discernible until after the globulose degenerating contents of the animal had been largely appropriated. Before this stage was reached filaments from the assimilative mycelium had usually grown out through the host integument and had begun to extend themselves over the surface of the substratum, at the same time giving rise in successively more distal positions to erect, flask-shaped, conidiiferous branches. Ultimately, when the filaments have concluded their development and have become partitioned at moderate intervals, the conidiiferous branches are to be found arising singly or, less often, in pairs from positions usually a short distance back of the cross-

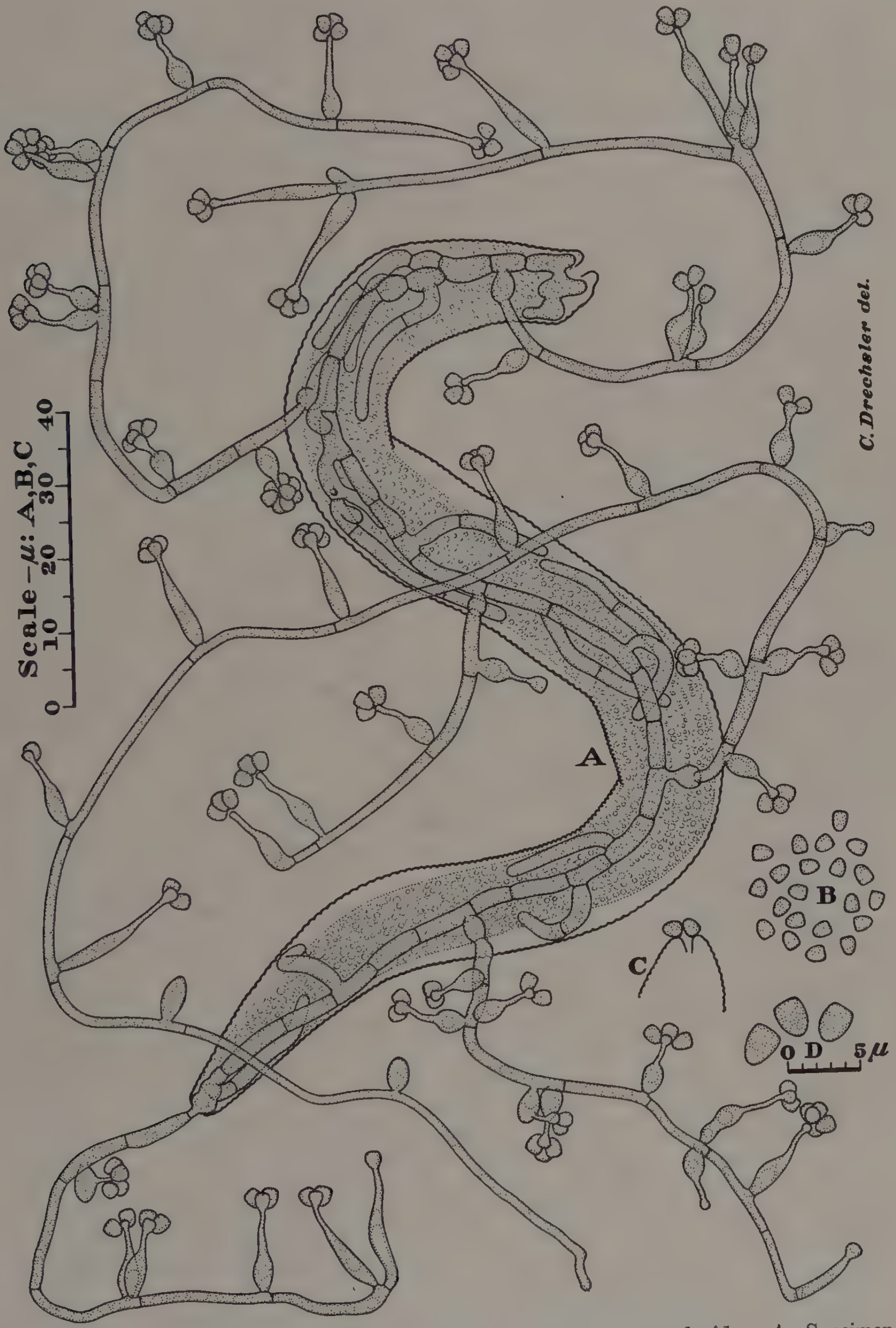
Scale – μ: A,B,C
0
10
20
30
40
A
B
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FIG. 4. *Cephalosporium balanoides*, drawn with aid of camera lucida. A. Specimen of *Acrobeloides bütschlii* from which 6 conidiophorous hyphae have grown out; not all of the conidia produced on the sterigmata are shown; ×1000. B. Detached conidia; ×1000. C. Two conidia affixed to head of a specimen of *A. bütschlii;* ×1000. D. Conidia; ×2000.

walls. The individual branch, as a rule, tapers distally into a slender sterigma, yet in some instances it is represented by an ellipsoidal body from which a sterigma arises laterally. Each sterigma abjoints successively about a dozen conidia, which cohere and for some time remain attached in a terminal head. During later stages of an epizootic within an agar culture conidia are often to be seen adhering to a host animal close to its mouth (Fig. 4, C), suggesting that frequently, if not usually, invasion may proceed from the head toward the tail.

In conformity to what would seem to be prevailing usage, I am referring the fungus to the genus *Cephalosporium*. It is hoped that this disposition, with its implication that the flask-shaped elements are interpretable as conidiophores, will not make for any misunderstanding of homologies with other nematode-destroying hyphomycetes. Obviously these branches are homologous not with the erect or ascending axial filaments in forms like *Acrostalagmus bactrosporus,* but with the flask-shaped branches borne on those filaments, in large part verticillately. The actual homologies might perhaps be brought into better relief by resorting to a somewhat recent terminology according to which the flask-shaped conidiiferous elements, regardless of their arrangement or position, are designated as phialides, while the filaments bearing them, whether erect or prostrate or submerged, are designated as phialophores.

A term meaning "like an acorn" is deemed suitable as an epithet for the fungus, though, considered more strictly, the conidia to which the term is intended to have reference, are invertedly nuciform, since they are truncate at the distal end and rounded at the proximal end (Fig. 4, B, D).

Cephalosporium balanoides, sp. nov.

Mycelium nutritum hyalinum, ramosum, septatum, intra vermiculos nematoideos evolutum, ex hyphis filiformibus 1.5–3 μ crassis constans. Hyphae fertiles extra animal emortuum evolutae, fere repentes rarius ascendentes, axe simplices vel parce ramosae, 50 μ–2 mm. longae, 1.2–2.5 μ crassae, in cellulis plerumque 10–30 μ longis consistentes, plerisque cellulis 1–2 ramulos conidiferos a fronte ferentibus; ramulis conidiferis (phialis) vulgo erectis, plerumque lageniformibus, 7–20 μ longis, prope basin 2–3.5 μ crassis, sursum vel rarius a latere sterigmate .5–.8 μ crasso praeditis, ex apice sterigmatis 5–15 conidia deinceps gerentibus; conidiis cohaerentibus, continuis, incoloratis, inversum nuciformibus, apice truncatis, basi rotundatis, 2.4–2.8 μ longis, circa 2.3 crassis.

Vermiculos nematoideos diversos necans habitat in humo silvestri prope Haugen, Wisconsin.

Assimilative mycelium hyaline, branched, septate, growing within living nematodes and composed of filamentous hyphae 1.5 to 3 μ wide. Conidiophorous hyphae developed outside of the dead host animal, usually creeping, rarely ascending, the axial filament simple or sparingly branched, 50 μ to 2 mm. long, mostly 1.2 to 2.5 μ wide, consisting of cells usually 10 to 30 μ long, most of which bear 1 or sometimes 2 conidiiferous branches at the distal end; the conidiiferous branches (phialides) commonly erect, mostly flask-shaped, 7 to 20 μ long, 2 to 3.5 μ wide near the base, bearing distally or laterally a sterigma .5 to .8 μ wide and at the apex thereof objointing 5 to 15 conidia one after another; conidia aseptate, colorless, invertedly nuciform, truncate at the distal end, rounded at the proximal end, 2.4 to 2.8 μ long, about 2.3 μ wide, cohering in a terminal head.

Destroying nematodes of several species, including *Plectus parvus* and *Acrobeloides bütschlii,* it occurs in leaf mold near Haugen, Wis.

A delicate and rather handsome fungus was found destroying young specimens of *Plectus parvus* in several maize-meal-agar plate cultures to which had been added small quantities of leaf mold collected near Butternut, Wis., in September, 1938. As the animals attacked were mostly of small size and in many instances were parasitized at the same time by a minute protozoan, the assimilative mycelium, though regularly extending from head to tail, often consisted only of a single meagerly branched hypha or of 2 such hyphae (Fig. 5, A, B, C). The reproductive filaments likewise were few in number. Where the host animal had happened to succumb on the surface of the culture medium these filaments assumed almost immediately an erect or ascending habit. Where, on the other hand, the host had succumbed under the medium, the reproductive filaments pushed their way to the surface, and extended ascendingly or gave rise to erect branches. On the aerial branches or prolongations flask-shaped conidiiferous ramuli were borne in handsome whorls; whereas the recumbent portions of the reproductive filaments bore such ramuli in erect posture and in promiscuous arrangement. The conidia, produced one after another on the ramuli, remained attached to form radiating or ascending chains (Fig. 5, A, B, C).

The fungus manifestly is referable to the genus *Spicaria,* within which it is distinguished by the small size and coccoid shape of its conidia (Fig. 5, D).

Spicaria coccospora, sp. nov.

Mycelium nutritum hyalinum, ramosum, septatum, intra vermiculos nematoideos evolution, ex hyphis filiformibus 1.5–3.5 μ crassis constans. Hyphae fertiles extra animal emortuum evolutae, ex magna parte erectae vel ascendentes, axe simplices vel parce ramosae, vulgo 25–200 μ longae, 1.5–2.5 μ crassae, septatae, subter septis ramulos conidiferos ferentes; ramulis conidiferis (phialis) saepius 3–5 verticillatis, lageniformibus, vulgo 6–12 μ longis, 1.8–2.5 μ crassis, quoque in sterigma circa .6 μ crassum abeunte et ex apice sterigmatis 10–20 conidia deinceps gerente; conidiis continuis, hyalinis, globosis, 1.3–1.7 μ diam., in catenulas digestis.

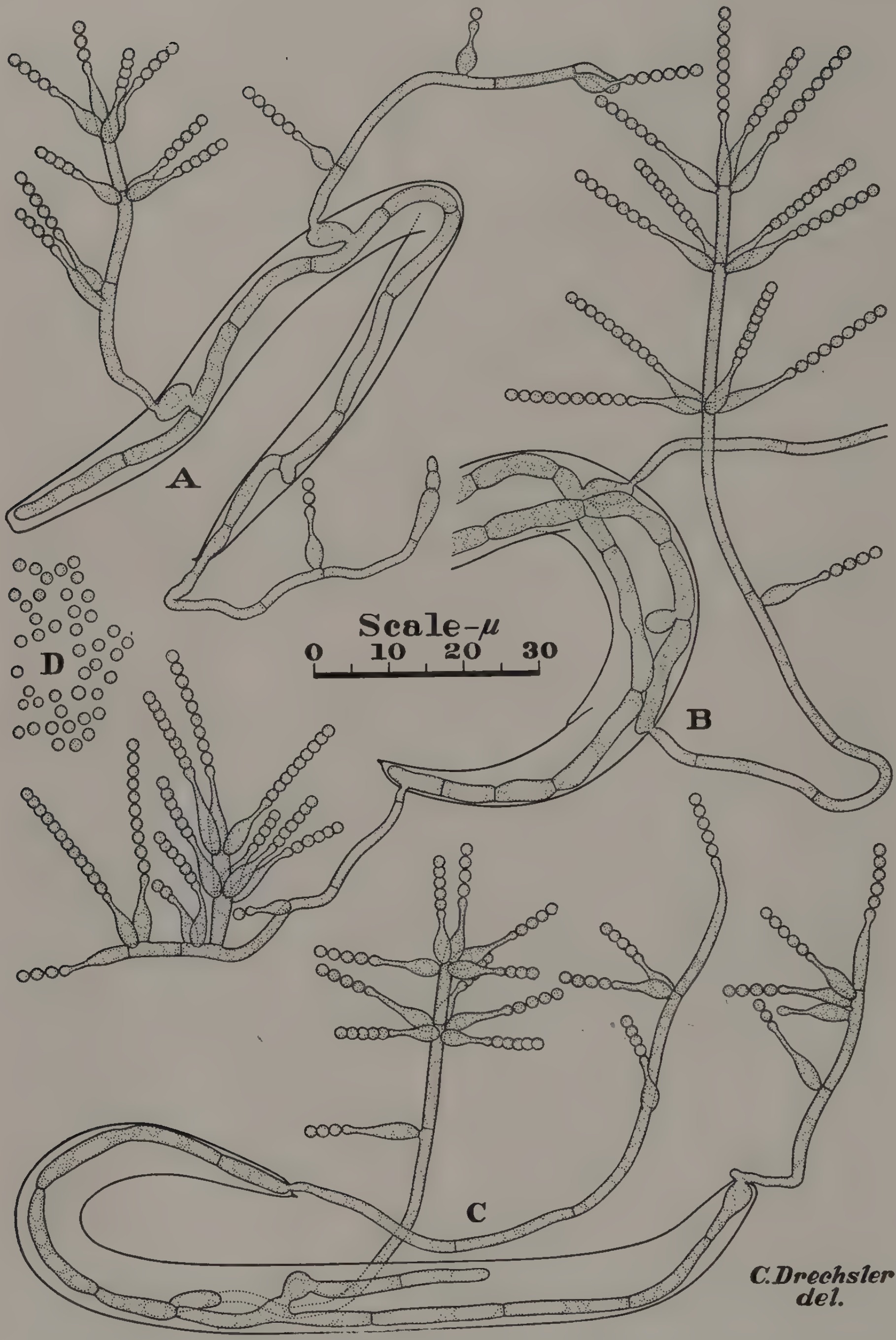
Plectum parvum necans habitat in humo silvestri prope Butternut, Wisconsin.

Assimilative mycelium hyaline, branched, septate, growing within living nematodes and composed of filamentous hyphae 1.5 to 3.5 μ wide. Conidiophorous hyphae developed outside of the dead host animal, largely erect or ascending, the axial filament simple or sparingly branched, commonly 25 to 200 μ long and 1.5 to 2.5 μ wide, septate, in positions just proximad of the septa bearing conidiiferous branches; the conidiiferous branches (phialides) often arranged in whorls of 3 to 5, flask-shaped, commonly 6 to 12 μ long and 1.8 to 2.5 μ wide, each terminating in a sterigma about .6 μ wide and at the apex thereof producing 10 to 20 conidia one after another; the conidia hyaline, aseptate, subspherical, 1.3 to 1.7 μ in diameter, arranged in chains.

Destroying nematodes of the species *Plectus parvus,* it occurs in leaf mold near Butternut, Wis.

A PARASITE WITH CONIDIOPHORES SOMEWHAT RESEMBLING TRANSVERSELY SEPTATE BASIDIA

A fungus possibly allied to the nematode-destroying species of *Acrostalagmus, Cephalosporium,* and *Spicaria,* though hardly to be considered congeneric with any of them, was observed in several old maize-meal-agar plate cultures to which had been added small quantities of leaf mold collected in Arlington, Va., late in November, 1937. In these cultures it subsisted largely on eelworms of the genus *Rhabditis,* often attacking specimens belonging apparently to *R. dolichura* (A. Schneider) Buetsch. Subsequently, the fungus was observed destroying individuals of the same sharp-


A
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Scale-μ
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C. Drechsler del.

FIG. 5. *Spicaria coccospora*, drawn to a uniform scale with the aid of a camera lucida; ×1000 throughout. A, B, C. Specimens of *Plectus parvus* occupied by assimilative mycelium, which has given rise externally to aerial conidial apparatus. D. Detached conidia.

tailed species in maize-meal-agar plate cultures to which had been added leaf mold collected near Butternut, Wis., early in September, 1938. M. B. Linford has informed me that a parasite similar to and probably identical with the one under consideration was observed by him among the organisms destructive to nematodes in Hawaii.

Infected eelworms become thoroughly permeated with a rather profusely branched assimilative mycelium composed of septate filiform hyphae. Though filled with densely granular contents, these hyphae are not clearly discernible until they have largely appropriated the globulose degenerating contents of the host. Before this advanced stage in the utilization of the animal is reached, numerous conidiophores have been pushed out here and there through the integument. These conidiophores produce spores freely both under and on the surface of the substratum. When developing in submerged positions (Fig. 6, D, a–i; E, a, b), they usually are shorter than when developing recumbently (Fig. 6, D, j; F–H). Whether submerged or prostrate, they early become divided by cross walls into segments of rather uniform length. Each of the segments gives rise, usually at its distal end, to a short, delicate sterigma, on the tip of which small conical conidia are formed one after another. Where the sterigma is under the substratum, the spores abjointed from it, often numbering about a dozen, remain closely grouped for extended periods. Possibly this grouping is attributable more to the support provided by the ambient than to cohesiveness. Condia produced on the surface of an agar substratum, where they get less support, and where, besides, they are exposed to much more frequent jostling by active eelworms, generally show little clustering. When fully formed the conidia are predominantly of conical shape, with broadly rounded base and bluntly rounded apex (Fig. 6, I), though many specimens terminate distally in a minute knob (Fig. 6, J). This knob appears rather similar to the small apical excrescence present in conidia of *Nematoctonus tylosporus*, and, like the latter modification, may play some part in fixing the spore to a prospective host. At all events the spore always adheres to a nematode by its narrower apical end, the broader basal end projecting into the air at an approximately right angle with the integument. When the eelworm moves through an agar medium the affixed conidium folds backward against the integument, but does not become detached. After an epizootic is well advanced in a culture 10 to 20 conidia may often be seen affixed to a single animal, most of them adhering to the head.

The type of conidial development here described would appear to be very unusual among the Mucedinaceae. Striking similarity in manner of sporulation is offered by a fungus that attacks European larch, *Larix decidua* Mill., causing a leaf cast more especially in young plants. From the stomata of leaves infected by this fungus short sporiferous hyphae emerge in crowded tufts. After the individual hypha has been divided by 1 to 3 cross walls, each of the delimited cells puts forth a single sterigma on which commonly a solitary spore is found supported. Vuillemin (41), who, in 1896, gave the

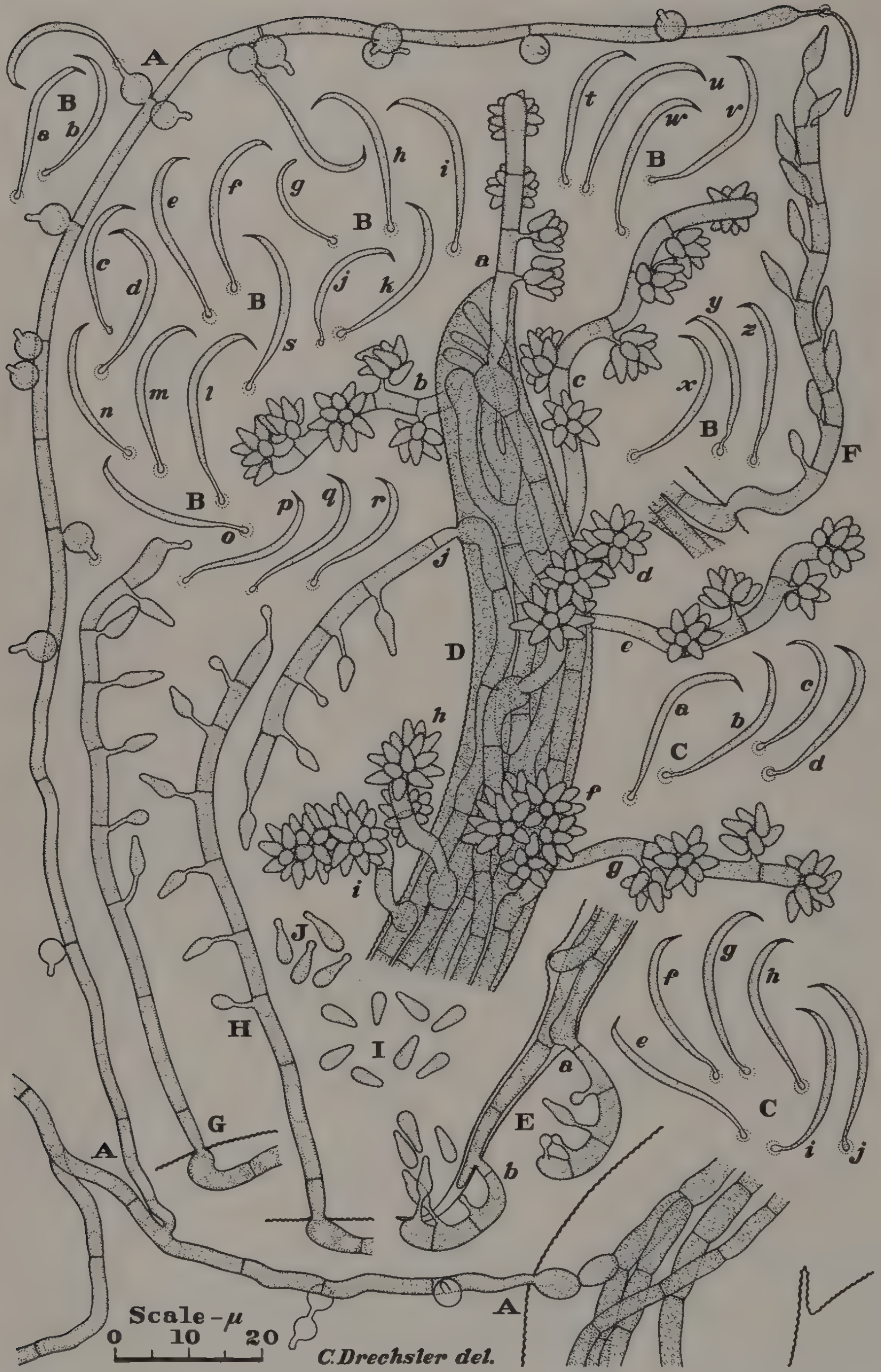
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FIG. 6. A–C. *Harposporium oxycoracum.* D–J *Meria coniospora.* For legend see opposite page.

first detailed account of the fungus, recognized the outward resemblance of the conidiophorus elements to promycelia of rusts. Further, he regarded the pseudoparenchymatous knots formed by the fungus within the stomatal chambers of the host—which knots, according to his account, give rise to the conidiophorus elements terminally on dichotomously branching outgrowths—as a distinctive type of fructification. Accordingly, in describing the parasite as a new species under the name *Meria Laricis* he not only erected on it a new genus, but made it the basis, in part, of a new family, the Hypostomaceae, which he placed "à la souche des Ascomycètes, vers le niveau d'où se détache l'ordre des Ustilaginées."

Apparently without knowledge of Vuillemin's publication, Hartig (14) 3 years later described the larch parasite as a new species, *Allescheria Laricis*, which, in view of the resemblance of its conidiophores to basidia of *Auricularia*, he made the basis of a supposedly new genus. However, since the term *Allescheria* had been used a little earlier by Saccardo and Sydow (34, v. 14, p. 464) as the generic name for a widely different fungus, the genus thus erected was compiled by Lindau (17) under a new name, *Hartigiella*, credited to Sydow. At this time, moreover, Lindau recognized the species as sole representative of a separate subfamily, the Hartigielleae, distinguished by the production of a single conidium on each cell of the conidiophore. Though Lindau (18, p. 260) later withdrew this subfamily in subsuming *H. Laricis* under the subdivision Botrydeae in the Mucedinaceae-Hyalosporae, he remarked that the method of spore formation in the species had scarcely any counterpart among the Hyphomycetes. In 1905 Vuillemin (42) pointed out the identity of Hartig's fungus with *Meria Laricis*, and took occasion to stress again his interpretation of the substomatal mycelial nodule as a peculiar fructification highly significant in the taxonomy of the fungus.

This interpretation cannot be held to have received much support from the more recent study of *Meria Laricis* by Peace and Holmes (29). When these investigators grew the fungus in pure culture it produced conidia on short septate conidiophores arising from hyphae thicker and more densely filled with protoplasm than ordinary mycelial hyphae. Since the conidiophores produced in pure culture were similar to the basidium-like elements

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FIG. 6. Drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout.

Harposporium oxycoracum: A. Portion of a nematode of the genus *Rhabditis* permeated with assimilative mycelium; this mycelium has put forth an external filament that has branched in giving rise to several conidiophorous hyphae, one of which is shown throughout its length. B, a–z; C, a–j. Random assortment of conidia, showing variations in size and shape.

Meria coniospora: D. Anterior portion of an infected specimen of *Rhabditis* permeated with assimilative mycelium from which 10 conidiophores have grown out, 9 of which, a–i, sporulated under the agar medium, while the tenth, j, was extended over the surface of the medium. E. Posterior portion of an infected specimen of *Rhabditis* containing assimilative hyphae from which 2 short conidiophores, a and b, have grown out over the surface of agar medium; one of them, a, consisting of 4 cells and thus presenting a specious resemblance to a transversely septate basidium. F, G, H. Conidiophores prostrate on the surface of agar medium. I. Detached conidia, showing variations in size and shape. J. Detached conidia, each with a distal knoblike modification.

developed externally on larch leaves under natural conditions, the stout hyphae from which they originated were justly considered as being probably equivalent to the substomatal mycelial masses found in nature—an equivalence strongly tending to abate the morphological significance that Vuillemin imputed to these masses. The correspondence of the conidiophores to transversely septate basidia likewise suffered some abatement from the further observation by Peace and Holmes that, in pure culture, the individual sterigma does not conclude its usefulness with the production of one conidium, but continues to form additional conidia, one after another. The British investigators held that probably also in nature an individual sterigma, on shedding one conidium, immediately starts to form another; whereas both Vuillemin and Hartig had presumed that each sterigma bore only a single spore. Though Peace and Holmes agreed with Vuillemin and with Hartig that the conidium contained no septum at the time of its abjunction, they noted that it laid down a median cross wall during an early stage in the process of germination.

Whether for purposes of taxonomy the cross wall thus laid down can be considered equivalent to the cross wall in a conidium normally uniseptate at the time of abjunction, appears uncertain. In some of the figures illustrating the germinating spores (29, Fig. 15, right; Fig. 16, right) one of the 2 chambers separated by the partition is shown devoid of contents. As the empty chamber in these instances bears no reproductive outgrowth of any kind, it must have lost its protoplasm by migration to the other chamber. Such movement of protoplasm, together with the early cessation of germinative development in the absence of nutrients, provides a rather marked analogy to the initial germination in conidia of *Nematoctonus tylosporus* and *N. leiosporus,* whereby these spores, though continuous at the time of abjunction, soon come to contain 1 (Fig. 1, P, a–c, e; Fig. 2, K, a–f, h) or even 2 (Fig. 1, P, d; Fig. 2, K, g) transverse partitions. With this analogy taken into account the findings of Peace and Holmes bring to light an extensive parallelism between the reproductive apparatus of *Meria Laricis* and that of the conical-spored fungus destructive to eelworms.

Production of spores on sterigmata arising singly from the segments of transversely septate conidiophores has been made known also in *Dixidium dixae.* However, owing in part to the swollen conformation of its sporiferous cells, and in part to the relatively large dimensions of its ellipsoidal spores, this curious entomogenous fungus presents an appearance rather little suggestive of the conical-spored parasite, from which it differs besides in its supplementary production of oidia. The parasite is, accordingly, referred to *Meria* rather than to *Dixidium,* though perhaps it may have closer affinities with the nematode-destroying species of *Acrostalagmus, Cephalosporium,* and *Spicaria* described herein than with *M. laricis,* to which, as well as to *D. dixae,* it is alien with respect to biotic relationship.

Meria coniospora, sp. nov.

Mycelium nutritum hyalinum, multum ramosum, septatum, intra vermiculos nematoideos evolutum, ex hyphis filiformibus plerumque circa 3 μ crassis constans, primo protoplasmatis dense granulosi repletum. Hyphae fertiles intra materiam ambientem vel super materiam subjacentem evolutae, hyalinae, simplices, plerumque 20–100 μ longae, saepius 2.5–3.5 μ crassae, vulgo 3–10-septatae, septis inter se 5–15 μ distantibus, cellula infima modo sterili modo fertili, aliis cellulis constantius fertilibus, quoque ex unico sterigmate 1.5–4 μ longo apice .6 μ crasso 5–15 conidia deinceps gerente; conidiis hyalinis, continuis, conoideis, 4–7 μ longis, prope basin 1.8–2.5 μ crassis, utrinque rotundatis, apice quandoque in tuberculum minutum abeuntibus.

Vermiculos nematoideos diversos necans habitat in humo silvestri prope Butternut, Wisconsin, et in Arlington, Virginia.

Assimilative mycelium hyaline, richly branched, septate, growing within living nematodes, consisting of filamentous hyphae often about 3 μ wide, filled during early stages with densely granular protoplasm. Conidiophorous hyphae formed outside of the dead host animal, either within the surrounding material or on the underlying material, mostly 20 to 100 μ long, often 2.5 to 3.5 μ wide, commonly divided by 3 to 10 septa at intervals of 5 to 15 μ, the lowermost of the segments thus delimited sometimes sterile and sometimes sporiferous, the other segments more consistently sporiferous, each producing successively 5 to 15 conidia on a single sterigma 1.5 to 4 μ long and about .6 μ wide at the tip; conidia hyaline, continuous, conical, 4 to 7 μ long, 1.8 to 2.5 μ wide near the base, rounded at both ends, sometimes terminating in a minute knob.

Destroying different nematodes, including species of *Rhabditis* it occurs in leaf mold near Butternut, Wis., and in Arlington, Va.

THREE PARASITES BELONGING IN THE GENUS HARPOSPORIUM

The fungus that under the binomial *Harposporium anguillulae* Lohde was first set forth in adequate detail by Zopf (43) appears far more frequently and is much more destructive than any other of the various hyphomyceous parasites found subsisting on nematodes in maize-meal-agar plate plate cultures prepared with decaying vegetable materials. Among the parasites encountered only occasionally are several forms that seemingly represent species distinct from the one discussed by Zopf, yet produce, likewise, relatively small crescent-shaped conidia on delicate sterigmata arising from globose excrescences borne laterally on conidiophorous filaments, and, therefore, likewise conform to Lohde's original description (21) of *H. anguillulae*. It is possible that utilization by different investigators of material belonging to separate species may underlie some of the discrepancies to be noted in the relevant literature,—such as come to light, for example, when the muticous filamentous conidia figured by Karling (15) and the similarly muticous, very slender, filamentous conidia figured by Sparrow (38) are compared with the acutely pointed conidia shown in Zopf's illustrations. Apart from forms with crescentic spores, 3 species have been observed that, in their parasitism and morphology, reveal general parallelism with *H. anguillulae*.

The most frequent of the 3 species has been found from time to time destroying nematodes in maize-meal-agar cultures of rather soft consistency, following the addition of leaf mold collected in deciduous woods near Beltsville, Md., near Cumberland, Md., and in Arlington, Va. It appears to attack eelworms of the genus *Rhabditis* somewhat more often than representatives of other genera. The globulose degeneration of fleshy parts that accompanies infection badly obscures the invading mycelium for some time. When the globulose materials have mostly been expropriated the assimilative mycelium becomes visible as a branching system of hyphae partitioned at rather close intervals into segments densely filled with coarsely granular protoplasm (Fig. 3, G). Usually, before this stage is reached, the mycelium has given rise, mostly laterally, to somewhat inflated parts; and each of these parts has put forth a narrow process, which, after erupting from the integument and perhaps widening perceptibly, has grown out as a conidiophorous hypha (Fig. 3, G, a–e). In instances where the host animal happened to succumb on the surface of the culture, the conidiophorous hyphae, often numbering more than a dozen, are extended as prostrate filaments. Conidiophorous hyphae from host animals that succumbed at a slight depth under the substratum frequently make their way to the surface and then elongate in creeping posture; though where a parasitized eelworm succumbs at a greater depth, some or all of the filaments growing out of it may complete their development under the surface.

Like the similar filaments of *Harposporium anguillulae,* the conidiophorous hyphae soon become divided by cross walls into segments of moderate lengths (Fig. 3, G, a). On each segment that participates in sporulation are borne usually 1 to 5 globose cells, somewhat larger than the correponding structures of *H. anguillulae.* These cells are provided with 1, 2, or 3 sterigmata, appreciably shorter than the single sterigma usual in *H. anguillulae.* Even more distinctive of the fungus than its curiously constituted conidiiferous branches are its conidia (Fig. 3, H, a–k), which are about twice as long as those of *H. anguillulae,* and disposed typically in a wide 3-dimensional helix. In well-developed spores three-quarters of a helical turn is usually described, the direction of coiling being always clockwise. The helical conformation here is not wholly antithetical to the crescentic conformation of the conidia produced by *H. anguillulae,* for, if these crescentic conidia are carefully examined, a slight tendency toward helicoid modification, likewise in the same direction as in hop stems, is often observable. When fully developed the spores of the fungus under consideration taper gradually toward both ends, which, in the main, are bluntly rather than acutely pointed, and reveal no local thickening of the enveloping wall. A droplet of mucus, secreted at the junction of sterigma and conidium, surrounds the basal end of the conidium after disarticulation.

The general resemblance of the parasite to *Harposporium anguillulae* warrants its description as a congeneric species, for which an epithet, meaning "of spiral form," may not be inappropriate.

Harposporium helicoides, sp. nov.

Mycelium nutritum hyalinum, ramosum, septatum, intra vermiculos nematoideos evolutum, in hyphis filiformibus plerumque 2–4 μ crassis constans. Hyphae fertiles, animali emortuo, intra materiam ambientem vel super materiam subjacentem evolutae, simplices vel rarius parum ramosae, mediocriter septatae, hyalinae, saepius 25 μ–1 mm. longae, 1–3 μ crassae, hic illic ramulos conidiferos (phialas) globosos plerumque 3.5–4.5 μ crassos ferentes; quoque ramulo 1–3 sterigmatibus 1–1.5 μ longis, circa .7 μ crassis sursum praedito; conidiis hyalinis, continuis, filiformibus, in tres partes spirae ad modum caulis *Humuli Lupuli* convolutis, utrinque attenuatis, basi et apice angustis sed tamen aliquid hebetibus, basi guttula muci vestitis, .7–1.5 μ crassis, in cursu curvato plerumque 20–45 μ longis.

Vermiculos nematoideos diversos necans habitat in humo silvestri prope Beltsville, Maryland, prope Cumberland, Maryland, et in Arlington, Virginia.

Assimilative mycelium hyaline, branched, septate, consisting of filamentous hyphae mostly 2 to 4 μ wide, growing within living nematodes. Conidiophorous hyphae after the death of the host animal formed in the surrounding material or on the underlying material, simple or more rarely slightly branched, hyaline, septate at moderate intervals, often 25 μ to 1 mm. long, 1 to 3 μ wide, here and there bearing globose conidiiferous branches (phialides) mostly 3.5 to 4.5 μ in diameter, each of which is provided distally with 1 to 3 sterigmata 1 to 1.5 μ long and about .7 μ wide; the conidia colorless, continuous, filiform, describing three quarters of a helical coil wound like stems of *Humulus Lupulus*, measuring .7 to 1.5 μ in greatest width, and 20 to 45 μ in length along the curved axis, tapering toward both ends, narrow but yet somewhat blunt at base and apex, at the base surrounded with a droplet of mucus.

Destroying various nematodes, including some species of *Rhabditis*, it occurs in leaf mold near Beltsville, Md., near Cumberland, Md., and in Arlington, Va.

Another fungus closely resembling *Harposporium anguillulae* was observed in numerous maize-meal-agar cultures to which had been added small quantities of leaf mold collected in deciduous woods near Butternut, Wis., in September, 1938. In these cultures it subsisted mainly by the destruction of large eelworms, often about 1 mm. in length, belonging apparently to a species of *Plectus;* and occasionally attacked, besides, nematodes referable to species of *Wilsonema* and *Bunonema*. The fungus shows general agreement with *H. helicoides* both in the assimilative mycelium by means of which it appropriates the fleshy substance of the host animal, and in the conidiophorous hyphae it extends mostly on the surface of the surrounding or underlying material (Fig. 6, A). Agreement is evident also with respect to the size of the globose protuberances formed laterally on the conidiophorous hyphae, but not with respect to the distribution of sterigmata, for the individual protuberance here, just as in *H. anguillulae,* usually puts forth only a single sterigma. The conidia, which are shorter than those of *H. helicoides,* reveal unusual distinctiveness in characteristic modifications at both ends (Fig. 6, B, a–z; C, a–j). At the proximal end a minute knob-like basal part, immersed in a droplet of mucus, tapers upward into a delicate flexuous connection, which then gradually widens out into the subcylindrical main portion of the spore. Distally, the subcylindrical portion shows increasing curvature and at the same time tapers perceptibly before it terminates abruptly in an acute, aduncate apiculum consisting apparently of solid wall material.

The curious modification at the proximal end of the conidium seems to have no counterpart among other hyphomycetes known to parasitize nematodes. It offers, however, a striking correspondence to the basal appendage present on the spore of the wholly unrelated *Euryancale sacciospora.* Though the correspondence does not extend to all details—the appendages of the zoopagaceous parasite are devoid of protoplasm internally and of mucus externally—it nevertheless is suggestive of similarity in function. Such similarity is all the more likely, since among the eelworms attacked by the hyphomycetous parasite is included the same species of *Bunonema* on which *E. sacciospora* subsists habitually. The rangy cephalic processes of this eelworm and the warty protuberances on its body might offer opportunity for entanglement of delicate flexible processes, and thereby give scope for possible operation of such processes in attaching conidia to a prospective host.

Unlike the pendulous modification at the proximal end of the conidium, the cuspidate modification at the distal end is not without a counterpart among the small number of closely allied parasites. As is evident from Zopf's figures of *Harposporium anguillulae* the conidia of that species are rather sharply pointed at the apex. In the very abundant material that, from agreement with Zopf's description, I have held referable to *H. anguillulae* the conidia likewise were consistently pointed, the apex often being represented by a minute cusp, much smaller and less narrowly acute than the apiculum in the fungus under consideration, though similar to that structure in being composed of solid wall material. According to an account by Kostka (16) the pointed conidial apex in *H. anguillulae* serves an important function by facilitating the operation of a special mechanism for affixing the spore to a prospective host. The mechanism was set forth as consisting of the globose cell together with the sterigma bearing the spore. When a nematode brushes against the conidium the sterigma allegedly is pushed out of its normal position, momentarily deforming the globose cell, which, being elastic, then reactively exerts an upward pressure on the spore with the result that the sharp apex is driven into the animal. The conidiophore of *H. anguillulae,* bristling with many units of the formidable mechanism, was vividly compared in its fearful operation with the sickle-bearing chariots of ancient times. Consonant with this imagery Kostka's figure of the conidiophore (16, Abb. 2, a), though obviously copied for the most part after one of Zopf's illustrations (44, p. 303, Fig. 23, XI), shows much more narrowly pointed conidia, as also shorter and more strongly tapering sterigmata than were described or pictured by Zopf either in his first account of *H. anguillulae* or in later passages relevant to the fungus.

It is not certain whether Kostka's novel account of infection was based on observation or on inference. In *Harposporium anguillulae* and in *H. helicoides,* the connection between the mature conidium and its supporting sterigma appears so lacking in firmness that effective transmission of mechanical energy from the globose element to the tip of the spore seems improbable. In the fungus from Wisconsin such transmission would presumably be further impeded owing to the flexible character of the basal portion of the spore. Nevertheless the cuspidate conidial apiculum of this fungus does have the appearance of being designed for hooking the spore to or into a prospective host,—a purpose that, conceivably, might be accomplished through mere impact of the nematode moving in its ordinary locomotion. It is hoped that an epithet meaning "with a sharp hook" will prove conveniently suggestive of the outward conformation resulting in part from the presence of the acute apiculum.

Harposporium oxycoracum, sp. nov.

Mycelium nutritum incoloratum, ramosum, septatum, intra vermiculos nematoideos viventes evolutum, in hyphis filiformibus plerumque 2–4 μ crassis constans. Hyphae fertiles post mortem animalis extra corpus ejus evolutae, vulgo repentes, plerumque simplices, rarius parum ramosae, incoloratae, septatae, saepius 25 μ–1 mm. longae, 1–3 μ crassae, hic illic ramulos conidiferos (phialas) globosos 3.5–4.5 μ crassos ferentes; quoque ramulo unico sterigmate 1.2–2 μ longo circa .8 μ crasso praedito; conidiis continuis, incoloratis, aduncis, in totum 15–25 μ longis, .7–1.4 μ crassis, deorsum usque .3–.4 μ attenuatis, basi tubere .5–1 μ crasso in guttula muci circumdato praeditis, sursum minus attenuatis, subito in cuspidem solidam acrem saepius 2–2.5 μ longam abeuntibus.

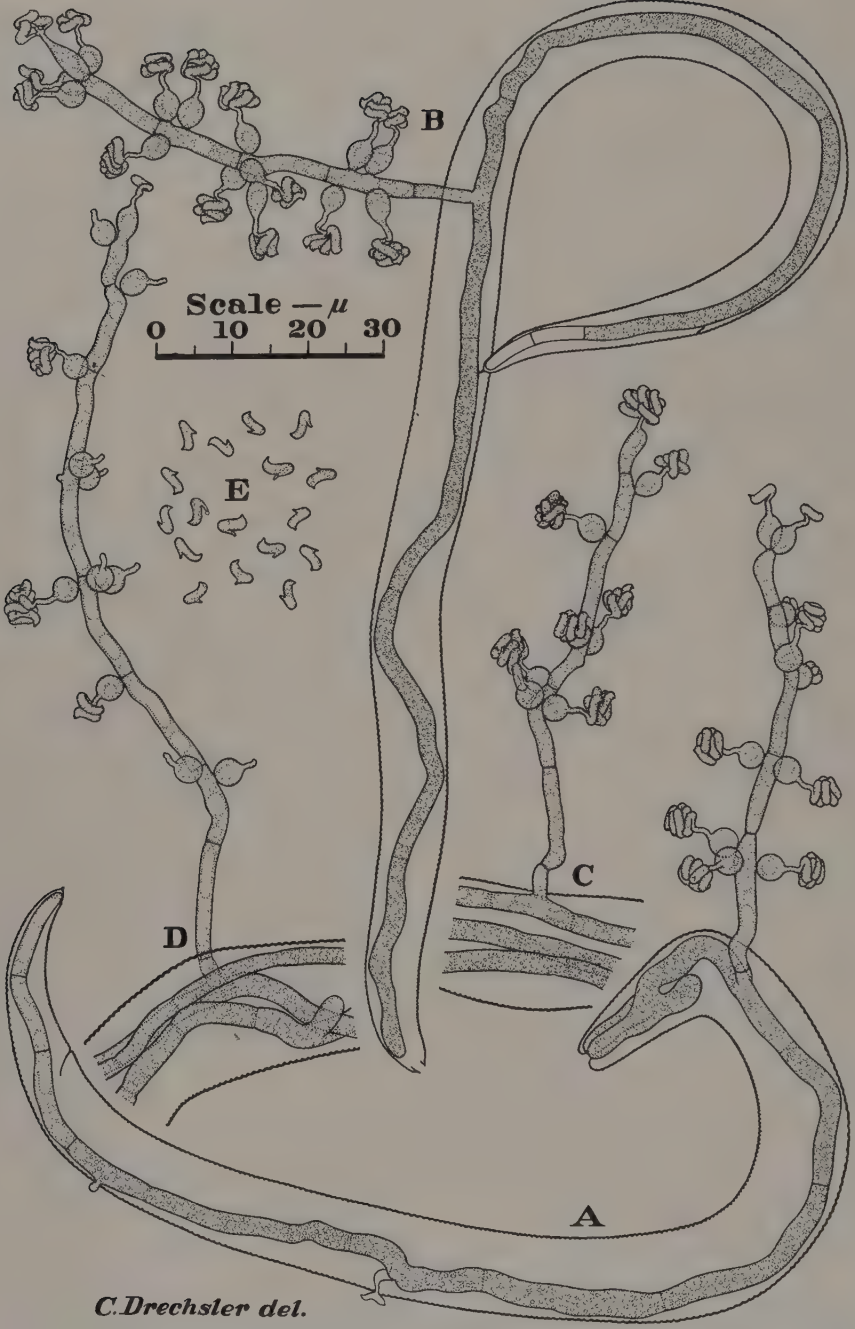
Vermiculos nematoideos generum Plecti, Wilsonematis, Bunonematis necans habitat in humo silvestri prope Butternut, Wisconsin.

Assimilative mycelium colorless, branched, septate, consisting of filamentous hyphae mostly 2 to 4 μ wide, growing within living nematodes. Conidiophorous hyphae formed externally after death of host animal, commonly prostrate, mostly simple, occasionally slightly branched, colorless, septate, often 25 μ to 1 mm. long, 1 to 3 μ wide, here and there bearing subspherical conidiiferous branches 3.5 to 4.5 μ in diameter, provided individually with a single sterigma 1.2 to 2 μ long and about .8 μ wide; conidia continuous, colorless, curved distally into hook-like form, measuring 15 to 25 μ in total length and .7 to 1.4 μ in greatest width, tapering downward to a width of .3 to .4 μ before expanding into a basal knob, .5 to 1 μ wide, immersed in a droplet of mucus, tapering upward less markedly to terminate abruptly in a sharp solid cusp often 2 to 2.5 μ long.

Destroying nematodes of the genera *Plectus*, *Wilsonema*, and *Bunonema*, it occurs in leaf mold near Butternut, Wis.

The third fungus resembling *Harposporium anguillulae* in a general way, yet not producing crescentic conidia, was observed in several maize-meal-agar cultures to which had been added small quantities of partly decayed leaves and stems of Kentucky blue grass, *Poa pratensis* L., collected in Arlington, Va., late in March, 1938. In these cultures it subsisted on nematodes, evidently to the exclusion of other nourishment. Most often it parasitized specimens of *Plectus parvus*. Invasion of small eelworms was accomplished often by the extension of a single assimilative filament from head to tail (Fig. 7, A, B), though several such filaments were usually operative in the destruction of larger animals (Fig. 7, C, D). Whatever their number the internal filaments always remained badly obscured until the globulose degenerating materials of the host had been largely appropriated by them. Before this stage was reached, coniodiophorous hyphae had been extended either into the agar medium or over its surface. These hyphae gave rise laterally to sessile subspherical protuberances closely resembling those of *H. anguillulae*, except possibly for a slightly greater tendency toward a prolate shape (Fig. 7, B). As in *H. anguillulae* the protuberances here regularly burgeoned forth only a single sterigma. Generally about a dozen conidia were produced at the tip of each sterigma, where they remained cohering in a rather compact mass until they were bustled by active nematodes, or otherwise subjected to physical disturbance. After their separation they could be made out as rather small bodies of an elongate utriform shape modified at base and apex by the presence of minute spurs pointing in opposite directions approximately at right angles to the spore axis (Fig. 7, E).

The production of plural conidia one after another at the apex of an individual sterigma is not at variance with the manner of sporulation in *Harposporium anguillulae*. In Lohde's brief original account of that species a sickle-shaped or crescentic spore was stated to grow out of the sterigma; no comment being made as to whether the process was subse-



C. Drechsler del.

FIG. 7. *Harposporium diceraeum*, drawn to uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Specimen of *Plectus parvus* invaded by an assimilative hypha coming from a conidium whose empty envelope is seen attached to the integument; the hypha has put forth a single conidiophorous filament. B. Specimen of *Plectus* sp. invaded by a single assimilative hypha, from which a conidiophorous hypha has been put forth. C, D. Conidiophorous hyphae arising from median portions of specimens of *P. parvus* invaded by several assimilative filaments. E. Detached conidia, showing variations in size and shape.

quently repeated. Zopf (43) remarked that the sterigmata of *H. anguillulae* abstrict conidia at their tips, without specifying whether a single spore or several spores were produced at each tip. None of his figures show more than one conidium attached to a sterigma. Karling in his account of development in *H. anguillulae* stated that he had never seen a globular cell form more than one conidium. Yet, in agar cultures, some conidiophorous hyphae of *H. anguillulae* that, because of their origin from deeply submerged eelworms are constrained to sporulate under the medium, show 3 or 4 or even 5 conidia borne on the tip of each sterigma. As a rule the conidia produced in agar are more uniformly of small size than those produced in the air on sterigmata arising from globose protuberances borne on prostrate conidiophorous hyphae; and very probably this predominantly small size tends toward increase in number of spores formed. Rather uniformly small conidia likewise have sometimes been found attached plurally to submerged sterigmata of *H. helicoides* and *H. oxycoracum*.

Since the fungus from grass detritus thus presents no serious morphological disagreement with *Harposporium anguillulae* it is described as a congeneric species under an epithet meaning "two-pointed."

Harposporium diceraeum, sp. nov.

Mycelium nutritum incoloratum, ramosum, intra vermiculos nematoideos viventes evolutum, in hyphis filiformibus singulis vel compluribus 1.5–4 μ crassis constans. Hyphae fertiles post mortem animalis extra corpus ejus evolutae, incoloratae, septatae, plerumque simplices rarius parum ramosae, saepius 25–150 μ longae, 2–3 μ crassae, hic illic ramulos conidiferos ferentes; ramulis conidiferis (phialis) globosis vel paulo elongatis, 3–4 μ crassis, in sterigma 1.5–3 μ longum .7 μ crassum abeuntibus, ex apice sterigmatis 10–15 conidia deinceps in capitulum gerentibus; conidiis hyalinis, continuis, late siliquiformibus, 3.5–4 μ longis, 1–1.5 μ crassis, utrimque rostratis; rostris minutis, directiangulis vel obliquis, binis inter se aversis.

Plectum parvum necans habitat in foliis caulibusque Poae pratensis putrescentibus in Arlington, Virginia.

Assimilative mycelium colorless, branched, septate, consisting of one or several filamentous hyphae 1.5 to 4 μ wide, growing within living nematodes. Conidiophorous hyphae formed externally after death of host animal, colorless, septate, mostly simple, occasionally somewhat branched, often 25 to 150 μ long, 2 to 3 μ wide, here and there bearing conidiiferous branches; these branches (phialides) subspherical or somewhat elongated, 3 to 4 μ in diameter, each provided distally with a sterigma 1.5 to 3 μ long and about .7 μ wide, at the tip of which 10 to 15 conidia are abjointed successively to cohere in a head; conidia hyaline, continuous, broadly pod-shaped, 3.5 to 4 μ long, 1 to 1.5 μ wide, minutely beaked at both ends; the 2 minute beaks perpendicular or oblique to the spore axis, and pointing in opposite directions.

Destroying *Plectus parvus* it occurs in decaying leaves and stems of *Poa pratensis* in Arlington, Va.

None of the 3 new species of *Harposporium* have so far been seen to produce spores other than the conidia attributed to them in the diagnoses. Nothing has come to light in material of any member of the genus that might be held to confirm the erroneous report of Sorokine (37) relative to production of minute zoospores in *H. anguillulae* (=*Polyrhina multiformis* Sor.)—a report amply refuted by Zopf, and subsequently further denied by Dangeard (6) and Karling. Although the chlamydospores that Zopf described for *H. anguillulae* are produced by that fungus no less consistently when it exterminates nematodes in agar cultures than when it destroys them in natural substrata, comparable reproductive bodies have not been observed in any material of the several congeneric forms. Survival through the resistant accessory spores probably accounts for the fact that, following addition of partly decayed vegetable detritus to nematode-infested agar cultures, *H. anguillulae* appears much more frequently than any of its congeners. In view of the presumptive advantage accruing from production of chlamydospores it is somewhat puzzling why this type of reproductive body is not more generally formed by the various hyphomycetes parasitic on nematodes. For that matter a like situation prevails in the series of hyphomycetes predaceous on nematodes, since of the 21 mucedinaceous forms known to capture eelworms in agar cultures (8, 11) only 4 have been observed to produce chlamydospores.

The similarity of the mucedinaceous parasite *Harposporium oxycoracum* to the zoopagaceous parasite *Euryancale sacciospora,* when considered in conjunction with the analogous similarity of the mucedinaceous predator *Dactylella bembicodes* Drechsl. (8) to the zoopagaceous predator *Stylopage hadra* Drechsl. (7), suggests that, despite pronounced dissimilarity in morphology of conidial apparatus, the genus *Harposporium* may possibly represent a group of parasitic forms closely related to the predaceous series of hyphomycetes. On the other hand, *H. diceraeum,* with its rather plump capitate conidia, offers features that might not unreasonably he held to connect the genus with the more commonplace nematode-destroying parasites that have been described herein as new species of *Acrostalagmus, Cephalosporium,* and *Spicaria,* and that may well be truly akin to entomogenous forms compiled in these genera. Certainly the subspherical protuberances of *Harposporium,* together with the single or plural sterigmata borne on them, must be held equivalent to the flask-shaped conidiiferous elements in *Acrostalagmus* and *Cephalosporium.* Such equivalence, indeed, would seem to have been presumed by Zopf when he designated the protuberances of *H. anguillulae* as basidia; for the term "basidium" was then not restricted, as it is now, to special structures in the Basidiomycetes, but was applied (1, p. 65; 44, p. 314) to outwardly modified lateral or terminal conidiiferous cells in fungi generally. In its more inclusive early meaning the term seems to have been broadly applicable to the variously differentiated conidiiferous structures that now are often designated as phialides.

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LITERATURE CITED

1. BARY, A. DE. Vergleichende Morphologie und Biologie der Pilze. 558 pp. Engelmann (Leipzig). 1884.
2. BUCHANAN, R. E. Morphology of the genus *Cephalosporium* with description of a new species and a variety. Mycologia 3: 170–174. 1911.
3. BULLER, A. H. R. Researches on fungi. 2: 492 pp. Longmans Green & Co. (London). 1922.

4. COUCH, J. N. A new fungus intermediate between the rusts and *Septobasidium.* Mycologia 29: 665–673. 1937.
5. ————. The genus *Septobasidium.* 480 pp. University of North Carolina Press (Chapel Hill). 1938.
6. DANGEARD, P. A. Memoire sur quelques maladies des algues et des animaux. Le Botaniste 2: 231–268. 1891.
7. DRECHSLER, C. A new species of conidial phycomycete preying on nematodes. Mycologia 27: 206–215. 1935.
8. ————. Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29: 447–552. 1937.
9. ————. Five new Zoopagaceae destructive to rhizopods and nematodes. Mycologia 31: 388–415. 1939.
10. ————. Three fungi destructive to free-living terricolous nematodes. Jour. Washington Acad. Sci. 30: 240–254. 1940.
11. ————. Three new hyphomycetes preying on free-living terricolous nematodes. Mycologia 32: 448–470. 1940.
12. GUÉGUIN, F. Les champignons parasites de l'homme et des animaux. XVII + 299 pp. A. Joanin et Cie. (Paris). 1904.
13. ————. *Acrostalagmus Vilmorinii* n. sp., Mucédinée produisant une maladie à sclérotes du collet des Reines-Marguerites. Bull. Soc. Mycol. France 22: 254–265. 1906.
14. HARTIG, R. Die Lärchennadelbräune, erzeugt durch *Allescheria Laricis* n. sp. Centralbl. Gesamm. Forstwesen 25: 423–426. 1899.
15. KARLING, J. S. *Harposporium Anguillulae.* Mycologia 30: 512–519. 1938.
16. KOSTKA, G. Nematodenfangende Pilze. Mikrokosmos 21: 151–156. 1927–1928.
17. LINDAU, G. Hyphomycetes. In Engler, A., and K. Prantl. Die Natürlichen Pflanzenfamilien. Teil I, Abt. 1**: 558–559. Engelmann (Leipzig). 1900.
18. ————. Die Pilze Deutschlands, Oesterreichs und der Schweis. VIII. Abteilung: Fungi imperfecti: Hyphomycetes (erste Halfte). In Rabenhorst, L. Kryptogamen-Flora. Ed. 2, Bd. 1, Abt. 8. 852 pp. Edward Kummer (Leipzig). 1904–1907.
19. LINFORD, M. B. Stimulated activity of natural enemies of nematodes. Science 85: 123–124. 1937.
20. ———— and F. YAP. Root-knot nematode injury restricted by a fungus. Phytopath. 29: 596–606. 1939.
21. LOHDE, G. Ueber einige neue parasitische Pilze. Tagebl. Versamml. Deut. Naturf. u. Aerzte 47: 203–206. 1874.
22. LYMAN, G. R. Culture studies on polymorphism of hymenomycetes. Proc. Boston Soc. Nat. Hist. 33: 125–209. 1907.
23. MACMILLAN, C. A probable new category of carnivorous plants. Bot. Gaz. 17: 381–382. 1892.
24. MASON, E. W. Annotated account of fungi received at the Imperial Mycological Institute, List II, Fascicle 2. 67 pp. 1933.
25. NOBLES, MILDRED K. Production of conidia by *Corticium incrustans.* Mycologia 29: 557–566. 1937.
26. OUDEMANS, C. A. J. A. Contributions a la flore mycologique des Pays-Bas, XVIII. Nederl. Kruidk. Arch. III, 2: 633–781. 1902.
27. PARKIN, J. Fungi parasitica on scale-insects (Coccidae and Aleurodidae): a general account with special reference to Ceylon forms. Ann. Roy. Bot. Gard. Peradeniya 3: 11–82. 1906.
28. PATOUILLARD, N. Une Clavariée entomogène. Rev. Mycol. 14: 67–70. 1892.
29. PEACE, T. R., and C. H. HOLMES. *Meria laricis,* the leaf cast disease of larch. Oxford Forestry Memoirs, No. 15. 29 pp. 1933.
30. PETCH, T. The genus *Trichosterigma* Petch. Trans. British Myc. Soc. 9: 93–94. 1924.
31. ————. Studies in entomogenous fungs—VI, *Cephalosporium* and associated fungi. Trans. British Myc. Soc. 10: 152–182. 1925.
32. ————. Notes on entomogenous fungi. Trans. British Myc. Soc. 16: 209–245. 1931.
33. POISSON, R. Sur deux entophytes parasites intestinaux de larves de diptères. Ann. Paras. Hum. et Comp. 10: 435–443. 1932.
34. SACCARDO, P. A. Sylloge fungorum. . . . 25 v. Patavii, Abellini. 1882–1931.
35. SARTORY, A. Champignons parasites de l'homme et des animaux. 895 + 47 pp. Lefrançois (Paris). 1920–1923.
36. SARTORY, A., and R. SARTORY. Les mycoses nouvelles, 4e Supplement aux Champignons parasites de l'homme et des animaux. 183 pp. Paris. 1936.
37. SOROKINE, N. Note sur les végétaux parasites des Anguillulae. Ann. Sci. Nat. Bot. VI, 4: 62–71. 1876.

38. SPARROW, F. K. Inoperculate chytridiaceous organisms collected in the vicinity of Ithaca, N. Y., with notes on other aquatic fungi. Mycologia 25: 513–535. 1933.
39. SPEARE, A. T. On certain entomogenous fungi. Mycologia 12: 62–76. 1920.
40. VANDENDRIES, R. La polarité sexuelle et le régime conidien. Bull. Soc. Myc. France 50: 202–212. 1934.
41. VUILLEMIN, P. Les Hypostomacées nouvelle famille de champignons parasites. Bull. Soc. Sci. Nancy, Series II, **14**: 15–67. 1896.
42. ————. Identité des genres *Meria* et *Hartigiella*. Annales Mycologici 3: 340–343. 1905.
43. ZOPF, W. Zur Kenntis der Infections-Krankheiten niederer Thiere und Pflanzen. Nova Acta Leop.-Carol. Deut. Acad. Naturf. **52**: 314–376. 1888.
44. ————. Die Pilze. *In* Schenk, A. Handbuch der Botanik. **4**: 271–781. Edward Trewendt (Breslau). 1890.

VARIATION IN CULTURE OF SEVERAL ISOLATES OF ARMILLARIA MELLEA FROM WESTERN WHITE PINE[1]

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INTRODUCTION

A survey of root diseases, conducted in northern Idaho on the Coeur d'Alene National Forest, showed that *Armillaria mellea* (Vahl et Fries) Fries is the most common local associate of root disease in western white pine, *Pinus monticola* Douglas (1). Tree-ring examination disclosed that certain of the trees probably had been infected for long periods prior to examination without serious reduction in increment. In other instances a greater amount of the root surface was infected with a consequently greater reduction in increment during the period of infection. It was impossible to determine from observation whether this variability was due to differences in susceptibility of the trees or in virulence of the fungus.

In view of the findings of this survey, it is obvious that an evaluation of the part played by *Armillaria mellea* in the decadence of coniferous stands in northern Idaho is desirable from the standpoint of forest management. Such an evaluation may be gained only from intensive investigation, the primary purposes of which would be to determine the relative pathogenicity of local "strains" of the fungus, the relative susceptibility of northern Idaho tree species of various ages and on various sites to fatal infection, and the possible effect of environmental factors on the virulence of the fungus.

The present study is devoted to the investigation of physiological variation in a number of isolates of *Armillaria mellea* cultured (most of them at the time of the survey) from infected roots of mature western white pines. Since the pathogenicity of all of these isolates has been demonstrated by their infection of living suscepts, and since the isolates were cultured from a single suscept species, it seemed probable that the degree of variation found in these isolates would not be so great as that in isolates of doubtful pathogenicity or in isolates cultured from a number of host species. Experiments were devised in which appearance in culture and rhizomorph development, rate of growth, degree of saprogenicity, and response of the tested isolates to differences in wood-moisture content, temperature, and pH were studied.

EXPERIMENTAL WORK

In the first experiment 27 isolates were grown in triplicate at room temperature on a malt-agar medium in Petri dishes. Rate of growth of the isolates was determined by periodic diameter measurements of the mycelium in each culture. Approximate number and length of rhizomorphs produced by each culture were periodically recorded. The results show a wide variation in mycelium, rhizomorph production, and rate of growth of the isolates. Certain of the isolates were characterized by a cottony, white, profuse mycelium with a considerable number of long rhizomorphs; others developed a very thin mycelium with no rhizomorphs, and still others formed a reddish-brown sclerotial mycelial mat with a considerable number of short rhizomorphs projecting into the agar from the lower side of the mat, as well as long rhizomorphs ramifying through the agar (Fig. 1). The replications of a single isolate, however, were found to have similar cultural characteristics. The amount of mycelial growth made by the isolates over a 30-day period varied from 38.5 mm. to 54.0 mm., while the mean differences in diameter between the 14th and 30th days ranged from 20.5 mm. to 32.0 mm.

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[1] Condensed from a thesis submitted by the senior author to the University of Idaho Graduate School in partial fulfillment of the requirements for the M.S. degree. Merrill E. Deters and Earle C. Blodgett were kind enough to criticize the manuscript.

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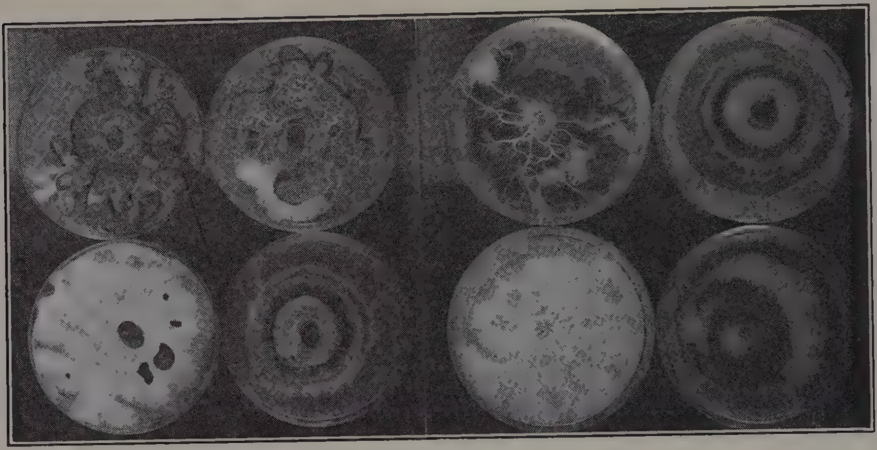
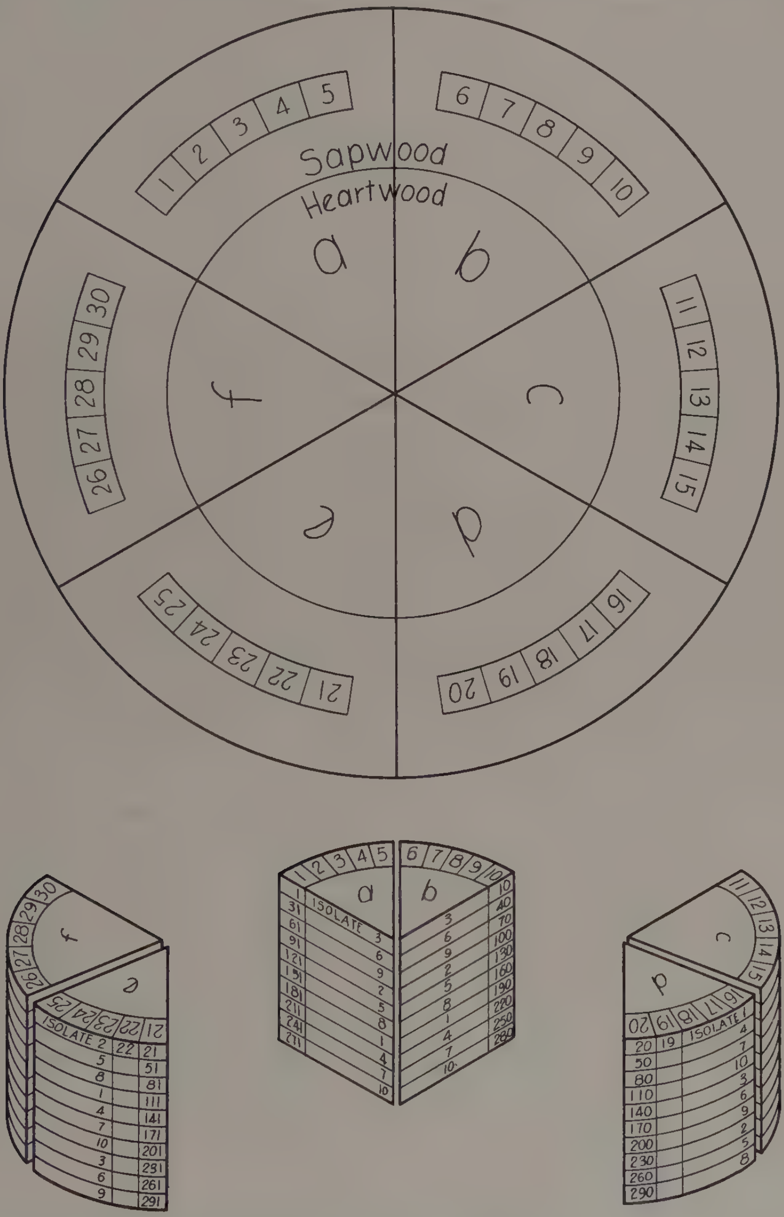


FIG. 1. Plate cultures of 4 isolates, showing differences in appearance of mycelium and rhizomorph production (left, upper surface; right, under surface).

In the second experiment test blocks of western-white-pine sapwood adjusted to 100 per cent initial moisture content (oven-dry basis), were exposed for 8 months to quintuplicate subcultures of 12 different isolates growing on a malt-agar medium in Parafilm-sealed extraction flasks. These isolates exhibited a wide variance in degree of saprogenicity, as judged by weight changes of the test blocks, which ranged from a mean gain of .98 per cent to a mean loss of 5.48 per cent (based on initial oven-dry weight).

In the third experiment 6 "matched" (Fig. 2) test blocks of western-white-pine sapwood, adjusted to each of 5 different initial moisture contents, were exposed (3 in each of 2 jars at a single moisture content) for 5 months at room temperature to subcultures of each of 10 isolates growing on a malt-agar medium in screw-capped jars. Percentage loss in weight of the wood blocks (initial oven-dry basis) was used as a measure of the degree of saprogenicity of the tested isolates. The results of this experiment (Fig. 3) indicate that a fairly wide difference in degree of saprogenicity exists between the most vigorous and least vigorous isolates (7.3 per cent loss in weight for isolate IX and 1.8 per cent loss in weight for isolate V). Average


Sapwood
Heartwood
a
b
c
d
e
f
1
2
3
4
5
6
7
8
9
10
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12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
ISOLATE

FIG. 2. Schematic drawing of log section, showing manner in which matched test blocks were cut and numbered for use in third experiment.

losses in weight range from .02 per cent for blocks at 50 per cent initial moisture content (isolate X) to 9.8 per cent for blocks at 100 per cent initial moisture content (isolate IX). Variance of isolates of *Armillaria mellea* cultured from roots of diseased western white pines is further illustrated by the differences in degree of saprogenicity of the isolates in this experiment.

Because of changes in moisture content of the blocks during the test period, it was not possible to determine within narrow limits the optimum moisture content for saprogenic activity of the tested isolates in western-white-pine sapwood. Figure 3, however, shows that the amount of decay effected by the isolates increased up to 150 per cent wood-moisture content. The maximum moisture content attainable in blocks of the specific gravity of western white pine, *i.e.*, .36 to .38 (2, p. 53), is approximately 200 per cent and the maximum air space attainable in wood of this specific gravity at

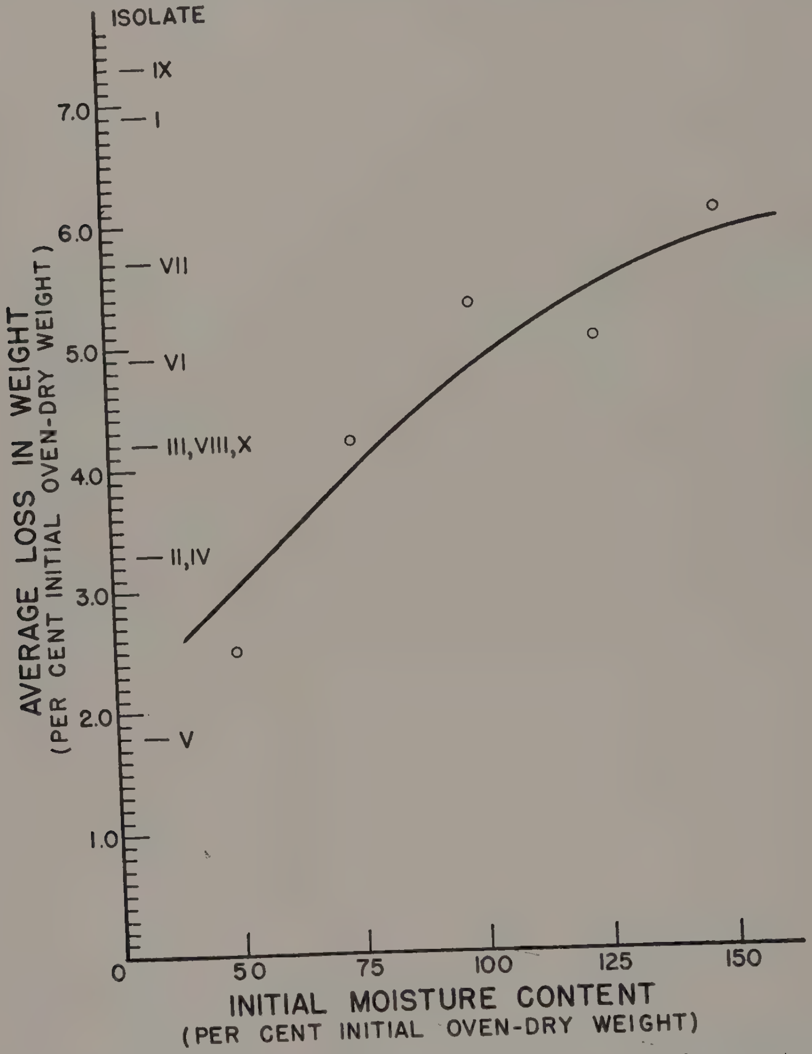

FIG. 3. Average losses in weight for all isolates at each initial moisture content and for each isolate at all initial moisture contents.

150 per cent moisture content is approximately 20 per cent (3, Figures 7 and 8). In the light of previous work (5) in which 200 per cent wood-moisture content was found to be inhibitory for the growth of many wood-decaying fungi, and (6) in which air space equivalent to 20 per cent of the volume of the test block was found to be necessary for growth of wood-decaying fungi, it seems unlikely that optimum wood-moisture content for the growth of *Armillaria mellea* in western-white-pine sapwood is far in excess of 150 per cent. The test blocks were not reweighed after sterilization, but it is probable that their initial moisture content was somewhat less than 150 per cent.

It is noteworthy that all isolates in this saprogenicity experiment displayed some rhizomorph development after the wood blocks were introduced into the jars, despite the fact that some of these isolates had not produced any rhizomorphs when grown in plate culture, and also that the greatest degree of saprogenicity was evident in those isolates having greatest rhizomorph development in plate culture. This is noteworthy, since successful inoculation of living suscepts is effected apparently only by rhizomorphs of the fungus.

The temperature relations of these isolates were studied in the fourth experiment. Ten isolates (same as used in the third experiment) were grown in triplicate, Parafilm-sealed Petri-dish subcultures on a malt-agar medium for thirty days at temperatures of 21, 25, 29, and 33 degrees C. Diameter measurements of the mycelial mats taken every 2 days, beginning on the ninth day after planting, served as criteria of the influence of temperature. The average diameter growth of all isolates between the initial and final measurements (9th and 30th days) was greatest (31.9 and 30.9 mm.) at 21 and 25 degrees, least (2.2 mm.) at 33 degrees. Average differences between individual isolates varied from 36.9 mm. (Isolate IV at 21°) to 0 (Isolates VII, VIII, and X at 33°) (Fig. 4). The results indicate that tests conducted at or near the optimum will produce only a slight variation in the growth of the tested isolates. Variation in growth was shown to be greater at the less favorable higher temperature of 29° than at the optima or at the still less favorable higher temperature of 33°. In other words, slightly supraoptimal temperatures bring out differences in thermal response between isolates more effectively either than optimal temperatures, or than nearly inhibitory temperatures that retard growth to a level so low that response of all isolates is sharply limited. It is noteworthy that Isolate II made little growth at 25 degrees, as compared with other isolates, whereas it made the greatest growth of all the isolates at the less favorable temperatures of 29° and 33°. Also, Isolate IV which made the greatest growth of all isolates at 21° grew next to least at 33°.

Most favorable temperatures for growth of all isolates were 21 and 25°. Rhizomorph production, although not periodically recorded, was greater at 21° than at all other temperatures. Although in the absence of tests at lower temperatures, it is possible that the optimum may be below 21°, the investigations of Wolpert (7) and Reitsma (4) show that 15, and 15–19 degrees, respectively, were less suitable for growth of the fungus than 25°. It may, therefore, be concluded that the optimum temperature for the growth of *Armillaria mellea* in plate culture lies between 19° and 25° and probably is between 21° and 25°.

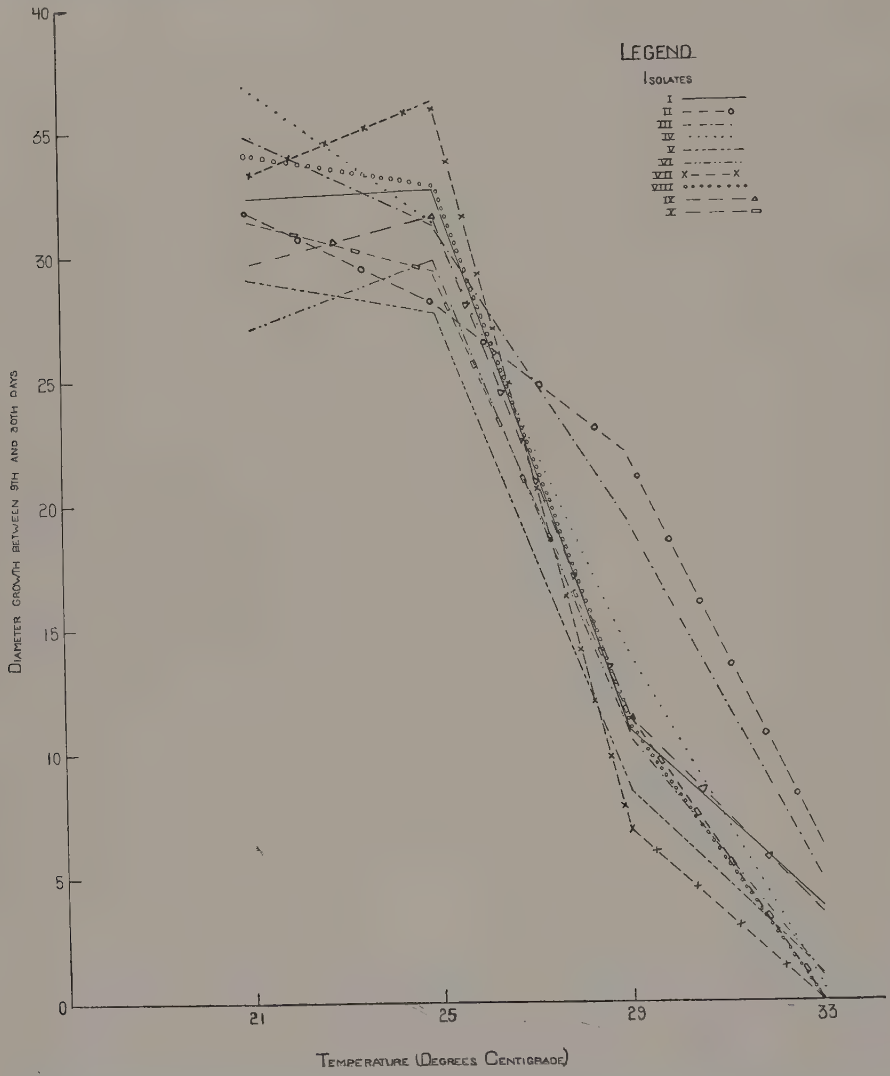

FIG. 4. Growth of 10 isolates of *Armillaria mellea* at different temperatures.

In the fifth experiment these ten isolates were grown at 25° C. in triplicate, Parafilm-sealed, Petri-dish cultures on a malt-agar medium adjusted electrometrically to 7 initial pH values (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0). Growth of the cultures was determined by diameter measurements of the mycelium taken every 2 days beginning on the 9th day after planting and ending on the 30th day after planting. The effect exerted by a slight difference in initial pH of the medium on the growth of the tested isolates is indicated by the marked differences in growth of isolates grown on media that varied by only .5 pH (4.0–4.5) (Fig. 5). In this experiment an approach to the lower pH limits resulted in reducing the range of differences between isolates, whereas, in the preceding experiment, a temperature somewhat above optimum resulted in an increase of the growth range. In these pH tests greatest range in growth occurred at the optimum initial pH, 4.5. Thus, at an initial pH of 4.5 the cultures of Isolate VII had an average measurement of 72.5 mm. on the 30th day, while, at the other extreme, Isolate IV had an average measurement at the same pH of only 46.5 mm.

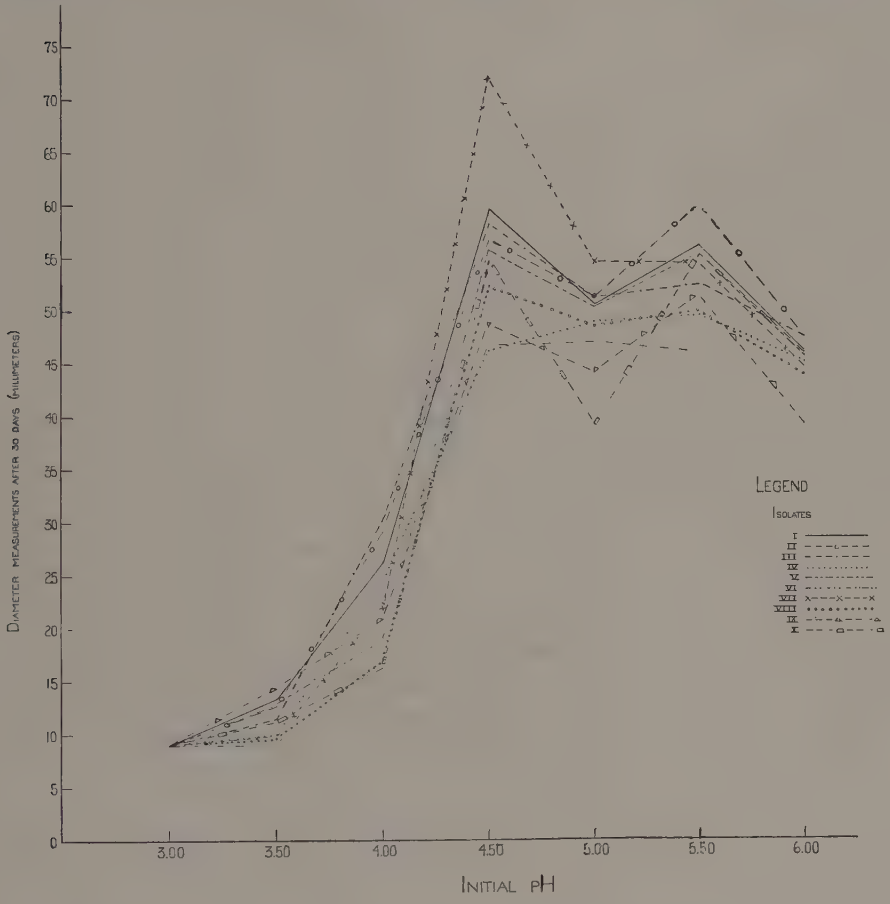

FIG. 5. Growth of 10 isolates of *Armillaria mellea* at different initial pH values.

Within the range of pH values and at the temperature (25° C.) of this experiment, it is evident that there are 2 optima for growth of the isolates, *i.e.*, pH 4.5 and pH 5.5. Existence of 2 optimal pH values for growth of this fungus also has been demonstrated by Wolpert (7) and Reitsma (4).

These results may be due to possible stimulation of growth at two points by the reagents used to adjust pH or to possible heterothallism of these mass isolates. Since the isolates grew vigorously at the highest pH values of this experiment, it was not possible to ascertain the supraoptimal inhibiting pH value. Wolpert, however, found that the upper pH limit for growth of the fungus at 25° is pH 6.9 in Richards' solution, and pH 7.8 in bacto-peptone solution.

A pH of 5.0 proved to be the most favorable for development of rhizomorphs by those isolates which normally exhibit rhizomorphic growth in plate culture. A pH of 3.0 was least favorable for the development of rhizomorphs.

DISCUSSION AND CONCLUSIONS

The results of the foregoing experiments demonstrate that the tested isolates vary in appearance in culture and rhizomorph production (Fig. 1), rate of growth, degree of saprogenicity (Fig. 3), and response to differences in wood-moisture content (Fig. 3), temperature (Fig. 4), and pH (Fig. 5). Repeated subculturing of these isolates showed them to be consistent in appearance of culture and production of rhizomorphs. Greatest degree of saprogenicity was evident in those isolates having the greatest rhizomorph development in plate culture. The similarity of response characteristic of certain isolates is illustrated by Isolates I and IX, which showed a close relationship in their response to different temperatures, and also displayed a close relationship in their degree of saprogenicity. In the saprogenicity tests these isolates made the greatest growth of all at the least favorable wood-moisture content (50 per cent) and grew best at the nearly inhibitory, suboptimal pH of 3.5, as well as ranking third in growth at the least favorable temperature (33°). Another example is Isolate V, which showed least saprogenicity of the isolates tested in the second and third experiments, and also made the smallest average growth of all isolates in the fourth experiment. A comparison of the results shown in figures 4 and 5 discloses that Isolates II and III, which made the greatest growth of all isolates at the nearly inhibitory temperature of 33°, also made the greatest growth at the less favorable pH 6.0. It is noteworthy that Isolate VII, which grew best of all isolates at optimum temperature (36.0 mm. at 25°) and at optimum pH (72.5 mm. at pH 4.5) grew not at all at the unfavorable temperature of 33° and next to least at the nearly inhibitory pH of 3.5, while Isolate II, which grew next least at the optimum temperature made the greatest growth of all isolates at 33° and ranked fourth in the amount of growth at pH 4.5. It may be concluded from these results (a) that isolates that are most vigorous under optimal conditions of temperature and pH may be expected to show slight vigor at the limits of these growth factors, and (b) that isolates showing little vigor at optimal temperature and pH may prove relatively more vigorous than any other isolates under unfavorable conditions of temperature and pH.

Since these tests were not repeated with the same isolates, they provide no absolute assurance of the constancy of these characteristics of the tested isolates. The fact, however, that replicate cultures of each isolate were used in all of these tests affords some evidence of the constancy of these characteristics.

Determination of these optimal values will facilitate production of inoculum for pathogenicity experiments.

SUMMARY

A number of western-white-pine isolates of *Armillaria mellea* have been studied for the purpose of determining the extent of physiological variation in culture. Five experiments were performed in which appearance in culture and rhizomorph production, diameter of mycelial growth, degree of saprogenicity, and response of the tested isolates to differences in wood-moisture content, temperature, and pH were determined. The results of these experiments demonstrate wide variations in cultural characteristics and response among certain of the tested isolates. The following optimal values were determined: approximately 150 per cent or slightly higher initial wood-moisture content (initial oven-dry basis), 21 to 25 degrees C. for growth on malt agar at pH 5.0, and pH 4.5 and 5.5 for growth on malt agar at 25°.

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LITERATURE CITED

1. EHRLICH, JOHN. A preliminary study of root diseases in western white pine. U. S. Dept. Agr., Forest Serv., Nor. Rocky Mt. Forest and Range Exp. Stat., Station Paper No. 1. 10 pp. 1939.
2. Forest Products Laboratory. Wood Handbook. Basic information on wood as a material of construction with data for its use in design and specifications. U. S. Dept. Agr., Forest Serv., Div. Forest Res. 326 pp. 1935.
3. MACLEAN, J. D. Manual on preservative treatment of wood by pressure. U. S. Dept. Agr., Misc. Publ. 224. 124 pp. 1935.
4. REITSMA, J. Studien über *Armillaria mellea* (Vahl) Quel. Phytopath. Zeitschr. 4: 461–522. 1932.
5. SNELL, WALTER H., NATHANIEL O. HOWARD and MYRON U. LAMB. The relation of moisture contents of wood to its decay. (II). Science **62**: 377–379. 1925.
6. ————. The relation of moisture contents of wood to its decay. (III). Amer. Jour. Bot. **16**: 543–546. 1929.
7. WOLPERT, F. S. Studies in the physiology of the fungi. XVII. The growth of certain wood-destroying fungi in relation to the H-ion concentration of the media. Ann. Mo. Bot. Gard. **11**: 43–97. 1924.

SOME RELATIONS BETWEEN MERCURIC CHLORIDE CONTENT, ACID CONTENT, AND FUNGICIDAL EFFICIENCY OF CERTAIN SOLUTIONS AS USED FOR POTATO–TUBER DISINFECTION

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INTRODUCTION

The control of rhizoctonia (*Corticium vagum* Berk. and Curt.) is one of the most important problems confronting the potato farmer. From 1925 to 1934, inclusive, rhizoctonia caused an estimated annual average loss of 2.6 per cent of the total potato crop of the United States (7, p. 8). The annual loss from rhizoctonia for this period in Maine was approximately 3.5 per cent, or 1,500,000 bu. In the same state, loss from this disease varies from a negligible amount to very destructive, according to the season. In some seasons 25 to 70 per cent of the plants in many fields are girdled by the fungus, resulting in unevenness of stand and reduction in acre yield. Furthermore, the buyers of seed potatoes are becoming more critical and are demanding seed stocks that are relatively free from the "black scurf" phase of rhizoctonia infection.

The disinfection of seed potatoes may reduce the damage by rhizoctonia. The process may also reduce seed-piece decay. Both results can increase the yield rate (6).

The standard long-time "soak" method (90 minutes in 1–1000 mercuric chloride solution, made up as 4 ounces to every 30 gallons of water) has been one of the most effective treatments applied in certain experiments (5 and 6). This treatment, however, has the disadvantage that it seems too time-consuming and tedious to many farmers. Further, when the solution is used it suffers a reduction in its $HgCl_2$ content (1, 4, 8, 9). There is no quick, satisfactory test for the farmer to determine the exact strength of the weakened solution. This lack makes it impossible for him to know exactly how much $HgCl_2$ to add to restore the solution to its original strength. It is now generally recommended that the solution be replenished by the addition of from $\frac{1}{2}$–1 oz. of $HgCl_2$ for each 4 or 5 bu. of potatoes treated, and that the solution be discarded after 4 or 5 lots of seed potatoes have been treated. To discard the entire solution after each treatment, in favor of a new one of full strength, is too expensive. Because of these difficulties, many farmers either fail to disinfect their seed potatoes or resort to easier and less effective methods. There is need of a seed-disinfecting method that is simple in application, noninjurious to the tubers, and effective in controlling disease.

Cunningham, in 1925 (2), found that the addition of a small amount of hydrochloric acid to the $HgCl_2$ solution greatly increased the effectiveness of the fungicide. Leach *et al.*, in 1929 (3), further tested the effectiveness of such an acidulated solution. They found that a 1–500 $HgCl_2$ solution (0.2 per cent), containing 1 per cent by volume of commercial HCl of about 31 per cent strength and employed as a 5-minute cold soak, gave virtually as good results as the 1–1000 $HgCl_2$ 2-hour-soak method. They showed further that the loss of $HgCl_2$ upon successive treatments was retarded by the addition of HCl to the solution.

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The experiments here reported were conducted to test further the merits of the acidulated-mercury method, and to improve it if possible.

GENERAL EXPERIMENTAL PROCEDURE

The first step in our experiments was to test, under laboratory conditions, the effect of the potato treatment on the $HgCl_2$ concentration of the treating solution comparing nonacidulated with acidulated solutions, 90-minute with 10-minute treatments, and distilled water with hard water as the solvent. Tests also were made for the loss of the acid used. This was followed by field tests in which the control of rhizoctonia by acidulated solutions was compared with control by nonacidulated. Here, also, samples were taken for laboratory analysis to determine the loss of $HgCl_2$ and acid, and here two kinds of acid were compared.

LABORATORY EXPERIMENTS

Each laboratory test was begun with 11 l. (2.906 U. S. gal.) of solution, which was used for 23 successive treatments applied, respectively, to 23 different lots of field-run unwashed Green Mountain potatoes, 7 lb. (3.175 kg.) per lot. A 250-cc. sample of the solution was taken for analysis at the beginning and after each treatment. When HCl was used, it was at the rate of 0.555 per cent by volume of the commercial form of about 31 per cent strength, equivalent to 0.2 per cent by weight. The hard water (pH 7.5), compared with distilled water as a solvent, was from Aroostook Farm in northeastern Maine, and contained calcium, magnesium, and sulphates according to qualitative tests. No chemicals were added to the solution following any treatment. The results of chemical analysis for $HgCl_2$ and HCl in relation to the various conditions of the tests are given in table 1.

The results in table 1 indicate that in nonacidulated solutions the $HgCl_2$ was depleted approximately 60 per cent by 90-minute treatments and about 27 per cent by 10-minute. Apparently, time length of treatment affected loss of strength, though not proportionately. In comparable acidulated solutions the $HgCl_2$ was depleted much more slowly than in the nonacidulated, to the extent of about 10 per cent by 90-minute treatments and about 9 per cent by 10-minute. The acid was depleted more rapidly than the salt. The longer treatments induced a somewhat greater loss of acid.

Incidentally it was observed that the nonacidulated solution became

TABLE 1.—*Concentration of mercuric chloride and hydrochloric acid, in solutions used for treating potatoes, in relation to the length of time of each treatment, the number of samples treated, and the pH of the water used in the preparation of the solutions*

Numerical order of treatment	Nonacidulated		Acidulated					
	Distilled water		Distilled water				Water with pH of 7.5	
	90 min.	10 min.	90 min.		10 min.		10 min.	
	Per cent $HgCl_2$	Per cent $HgCl_2$	Per cent $HgCl_2$	Per cent HCl	Per cent $HgCl_2$	Per cent HCl	Per cent $HgCl_2$	Per cent HCl
0	.100	.101	.201	.226	.202	.218	.202	.208
1	.096	.099	.202	.219	.202	.215	.203	.201
2	.096	.099	.202	.219	.202	.211	.203	.201
3	.095	.098	.202	.219	.202	.208	.203	.201
4	.093	.097	.202	.215	.202	.208	.203	.201
5	.091	.096	.202	.212	.201	.208	.204	.193
6	.089	.096	.201	.212	.202	.204	.202	.193
7	.086	.094	.201	.212	.201	.204	.203	.197
8	.084	.094	.199	.212	.200	.208	.202	.193
9	.082	.092	.198	.208	.200	.208	.201	.197
10	.079	.091	.198	.208	.199	.204	.202	.193
11	.076	.089	.197	.201	.198	.200	.200	.193
12	.072	.088	.197	.201	.198	.200	.200	.190
13	.068	.087	.195	.201	.198	.197	.199	.190
14	.067	.087	.195	.197	.196	.197	.198	.186
15	.065	.085	.193	.197	.195	.197	.197	.186
16	.061	.084	.192	.197	.196	.197	.197	.182
17	.058	.082	.190	.197	.194	.197	.196	.182
18	.055	.081	.188	.193	.192	.197	.194	.182
19	.053	.079	.186	.186	.191	.189	.192	.182
20	.050	.079	.185	.182	.190	.186	.191	.179
21	.047	.077	.183	.179	.189	.186	.190	.179
22	.044	.076	.181	.179	.186	.182	.187	.171
23	.040	.074	.180	.171	.184	.178	.186	.171
Total loss[a]	60.0	26.7	10.4	24.3	8.9	18.3	7.9	17.8

[a] As relative percentage lost through all 23 treatments.

exceedingly turbid and cleared very slowly, while the soil and other insoluble foreign materials from the tubers settled rapidly in the acidulated solution.

The depletion of acid and chloride, in treatments for the same length of time, was about the same in the solution made up with the hard water as in that with distilled water.

FIELD TESTS

In the field tests the solution was filled each time with Irish Cobbler tubers that were well coated by rhizoctonia sclerotia. The percentage of plants free of rhizoctonia lesions usually was ascertained from the examination of 100 7-inch plants for each treatment. In 1937, however, the test of effectiveness of the acidulated solution consisted of determining the viability of 20 sclerotia from each treated lot of tubers on potato-dextrose agar. The results of chemical analysis for $HgCl_2$, HCl, and acetic acid in relation to the various conditions of the test and in relation to the effectiveness of the solution against rhizoctonia infection of stems are given in table 2.

TABLE 2.—*Concentration of mercuric chloride, hydrochloric acid, and acetic acid, in solutions*[a] *used for treating potatoes, in relation to the length of time of each treatment, the number of samples treated, and the subsequent control of Rhizoctonia*

Numerical order of treatment	Nonacidulated		Acidulated									
	1937		1937[b]		1938			1939				
	90 min.		5 min.		5 min.			5 min.			5 min.	
	Per cent $HgCl_2$	Per cent plants free of lesions	Per cent $HgCl_2$	Per cent HCl	Per cent $HgCl_2$	Per cent HCl	Per cent plants free of lesions[c]	Per cent $HgCl_2$	Per cent HCl[d]	Per cent plants free of lesions	Per cent $HgCl_2$	Per cent $CH_3 \cdot COOH$[e]	Per cent plants free of lesions
0	.088	27[f]	.193	.193	.209	.190[g]	19[f]	.243	.511	17[f]	.217	1.74[h]	19[f]
1	.085	97	.206	.179	.201	.204	80	.242	.511	91	.215	1.80	89
2	.078	97	.207	.175	.206	.190	69	.241	.511	73	.214	1.80	89
3	.047	94	.209	.171	.205	.188	75	.239	.492	94	.215	1.80	89
4	.044	89	.208	.164	.207	.182	82	.239	.492	79	.214	1.77	95
5	.041	89	.210	.160	.211	.175	62	.236	.492	96	.214	1.77	91
6	.038	79	.210	.153	.208	.173	82	.236	.474	94	.212	1.74	89
7	.022	69	.210	.150	.209	.173	62	.234	.474	90	.213	1.77	87
8	.015	61	.211	.150	.211	.164	73	.232	.474	89	.210	1.80	87
9	.008	56	.210	.146	.215	.153	65	.233	.456	86	.210	1.77	83
10	.006	38	.209	.146	.215	.150	62	.231	.474	99	.210	1.77	93
11	.005	31	.209	.146	.217	.146	82	.231	.474	90	.210	1.74	89
12			.211	.142	.216	.142	58	.228	.438	93	.209	1.74	75
13			.206	.131	.212	.139	79	.227	.456	85	.209	1.71	95
14			.209	.120	.209	.135	72	.225	.456	86	.207	1.74	92
15			.212	.109	.212	.131	75	.222	.438	89	.207	1.74	95
16					.210	.120	71	.220	.438	93	.207	1.74	94
17					.209	.128	77	.218	.420	98	.204	1.71	93
18					.208	.117	86	.218	.420	93	.204	1.71	95
19					.207	.120	78	.215	.420	64	.202	1.71	72
20					.205	.113	77	.212	.420	83	.204	1.68	78
21					.201	.113	76	.210	.401	82	.201	1.71	95
22					.202	.113	76	.209	.401	82	.200	1.71	90
23					.196	.109	72	.207	.401	93	.200	1.71	90
24					.196	.102	91	.206	.383	98	.200	1.71	86
25					.197	.102	73	.203	.383	89	.200	1.71	87
Total loss[c, i]	94.3		None	43.5	5.7	46.3	74.2	16.5	25.0	88.4	7.8	1.7	88.7

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[a] Made up with the hard water tested with results given in table 1.

[b] With rhizoctonia sclerotia from treated tubers all nonviable, when tested on potato agar, in all but the 6th treatment, where a few germinated weakly.

[c] Treated controls, 90 min. in nonacidulated 0.1 per cent solution, were 90 per cent free of lesions.

[d] With pH of 1.15 at beginning and also after the 25th treatment.

[e] With pH of 3.1 at the beginning and also after the 25th treatment.

[f] Non-treated control tubers.

[g] Equivalent to 0.555 per cent by volume of commercial glacial acetic acid (approx. 31 per cent).

[h] Equivalent to 1.66 per cent by volume of glacial acetic acid.

[i] As relative percentage loss in percentage of concentration through all 11, 15, or 25 treatments.

From the results in table 2 it is apparent that, as in the laboratory tests, the $HgCl_2$ was depleted more rapidly in the nonacidulated solution than in the acidulated solutions. In the nonacidulated solution the $HgCl_2$ was depleted more rapidly than in the corresponding laboratory test, probably because of the use of more tubers (bearing more soil) in proportion to the solution in each treatment. In the nonacidulated solution the effectiveness against rhizoctonia infection of stems decreased with the decrease in mercury concentration, especially after treatment of the fifth lot, until only 31 per cent of the plants were free of lesions. In the acidulated solutions the effectiveness, while not up to that shown by the nonacidulated solution in the first treatment, remained high throughout the series of 25 treatments. This was true whether acidulation was secured with hydrochloric or acetic acid. The acetic acid was depleted much less than the hydrochloric. The test of the sclerotia on agar indicated a much greater effectiveness than was found in the stem-lesion test. This discrepancy had been noted in previous tests. Further, it was easier, more practical, and more reliable statistically to judge by stem lesions than by cultured sclerotia.

The average percentage of lesion-free plants for the 25 treatments with a solution acidulated with HCl was 74.2 in 1938 and 88.4 in 1939. The difference was attributable to the greater concentration of $HgCl_2$ in 1939, although the HCl concentration also was higher. The average percentage of lesion-free plants for the 25 treatments with solution acidulated with acetic acid was 88.7, although the $HgCl_2$ concentration was less than in the solution acidulated with HCl the same year and gave slightly less control.

As in the laboratory tests, turbidity was essentially eliminated by acidulation.

DISCUSSION

The experiments reported in this paper confirm the conclusions of other investigators that the amount of $HgCl_2$ in a solution is rapidly weakened by successive 90-minute treatments of potato tubers, unless the solution be acidulated. As a result of acidulation, the solution is effective for rhizoctonia control for 25 successive treatments instead of for only 5 or 6.

The data secured by the writers show also that the length of treating period may influence appreciably the rate of loss of $HgCl_2$ in the nonacidulated but not in the acidulated solution, when unwashed potatoes are treated. The length of time of treatment with acidulated solutions may be increased from 5-minute to 10-minute or even 90-minute periods with little reduction in the mercurial content and efficiency of the solution. This may be important in view of the fact that the 5-minute treatment with the acidulated solution has sometimes been somewhat less efficient in controlling rhizoctonia than has the 90-minute, nonacid treatment.

The good results secured by the use of acetic acid instead of HCl for acidulating the treating solution is important because the acetic acid is less injurious than HCl to human skin and clothing.

The fact that the acidulated $HgCl_2$ solution clears up after successive treatments is, in practice, considered in its favor.

Maine potato growers frequently have found both the nonacidulated and HCl-acidulated solutions injurious to tubers. The writers, however, have experienced no such injury provided the tubers be dried soon after treatment. Possibly the acetic acid solutions would be less injurious in practice.

SUMMARY

Rhizoctonia causes serious potato-crop losses. These losses can be reduced by seed disinfection. More farmers would disinfect their seed potatoes were as effective a solution available as is the standard cold 1–1000 $HgCl_2$, but requiring less time for each immersion of the tubers and retaining its effectiveness longer.

In laboratory experiments, the rapid lowering of $HgCl_2$ concentration through successive treatments of unwashed potato tubers was much retarded by shortening the duration of each treatment and by acidulating the solution with HCl, but not by shortening the duration of each treatment in the acidulated solution. The reduction of $HgCl_2$ concentration in the acidulated solution was not affected by the use of hard rather than distilled water as a solvent. Acidulation reduced considerably the turbidity of the treating solution. The acid was depleted more rapidly than the salt in the same solution.

Similarly, in field tests, there was a rapid lowering of $HgCl_2$ concentration and of effectiveness of rhizoctonia control; such changes were retarded by acidulation with HCl or acetic acid. The acetic acid was depleted much more slowly than the hydrochloric. Again, acidulation reduced turbidity.

The results indicate that $HgCl_2$ solutions intended for disinfecting seed potatoes should be acidulated, preferably with acetic acid.

LITERATURE CITED

1. Brann, J. W., and R. E. Vaughan. Potato scab. Wis. Agr. Exp. Stat. Bull. 331. 1921.
2. Cunningham, G. H. Corticium disease of potatoes. Experiments in control. N. Z. Jour. Agr. **30**: 14–21. 1925. (As abst. in Rev. Appl. Myc. **4**: 438–439.)
3. Leach, J. G., H. W. Johnson, and H. E. Parson. The use of acidulated mercuric chloride in disinfecting potato tubers for the control of Rhizoctonia. Phytopath. **19**: 713–724. 1929.
4. Orton, W. A. Selection and treatment of seed potatoes to avoid diseases. U. S. Dept. Agr., Bur. Plant Ind., Cotton, Truck, and Forage Crop Dis., Cir. 3. 1918.
5. Raleigh, W. P., and Reiner Bonde. Seed-potato treatment for Rhizoctonia control in northeastern Maine, 1929 to 1933. Phytopath. **26**: 321–343. 1936.
6. Schultz, E. S., L. O. Gratz, and Reiner Bonde. Effect of seed-potato treatment on yield and Rhizoctonia in northeastern Maine from 1925 to 1928. Phytopath. **20**: 47–64. 1930.
7. United States Department of Agriculture. Plant Disease Rptr. Supplement 89. 1935.
8. Weimer, J. L. Reduction in the strength of the mercuric-chloride solution used for disinfecting sweet potatoes. Jour. Agr. Res. [U. S.] **21**: 575–587. 1921.
9. White, R. P. Loss of strength of mercuric chloride solutions used for treating potatoes. (Abst.) Phytopath. **14**: 58. 1924.

SOIL TREATMENTS WITH VOLATILE LIQUIDS FOR CONTROL OF NEMATODES

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INTRODUCTION

Chloropicrin is now in widespread use for sterilization of greenhouse soils. In 1938 work was begun on the testing of varied doses and spacings of chloropicrin, as well as many other volatile liquids, against nematodes, or eelworms, in soil. Several chemicals showing the most promise were recorded in 1938[2] and 1939.[3] These included chloropicrin, butyraldehyde, crotonaldehyde, mesityl oxide, carbon disulphide, ethylene chloride, carbon tetrachloride, methyl n. amyl ketone, and mixtures of chloropicrin and ethylene chloride, or carbon tetrachloride. Results attained in 1939 eliminated butyraldehyde from further consideration.

In this article the results of tests conducted in 1939 and 1940 are reported. The experiments were designed primarily to determine the value of such substances in the treatment of soil, before planting, for the control of the nematodes *Ditylenchus dipsaci* (Kühn) Filipjev and *Heterodera marioni* (Cornu) Goodey. Unless otherwise specified, all injections of the chemicals were made with holes 10.5 in. and rows 9 in. apart[4] to a depth of 6 to 8 in. in well cultivated soil of a moisture content of 30 to 40 per cent of saturation. Such soil is easily molded in the hand but is not wet. After injection the soil surface was thoroughly moistened 2 or 3 times to a depth of 1 to 2 inches. These are considered the standard and most effective procedures. Using wet burlap sacking as a cover after moistening is a more recent improvement, used in a test on soil planted to tomatoes.

EXPERIMENTS ON THE CONTROL OF DAFFODIL EELWORM

One experiment was conducted outdoors on sandy loam infested with the daffodil strain of the nematode, *Ditylenchus dipsaci,* using four plots of 100 bulbs for each of the 8 treatments including controls (soil injected to a depth of 6 in.). The randomized-block method was used. Prior to planting, the bulbs were treated for 4 hr. in 0.5 per cent Formalin at 110° F. This bulb treatment is known to be highly efficient for the control of *D. dipsaci.*

The mean percentage of diseased bulbs in the controls was 35.075. Considering this as 0 per cent effective, the mean percentage efficacy of any treatment was obtained by dividing the difference between the percentage

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[1] The writer is indebted to Mr. L. B. Reed of the U. S. Bureau of Entomology and Plant Quarantine for aid in the statistical analysis.

[2] J. Parasit. **24**: 34. 1938.

[3] Third Internat. Congress for Microbiology (Sept. 2–9): 167–170. 1939.

[4] Taylor, Proc. Helm. Soc. Wash. **6**: 62–66. 1939.
Chitwood, Proc. Helm. Soc. Wash. **6**: 66–73. 1939.

TABLE 1.—*Ditylenchus dipsaci in narcissus planted in chemically treated soil. Experiment A*[a]

Chemical	cc. per injection	Per cent diseased bulbs in					Percentage efficacy	Approx. cost per acre
		Block A	Block B	Block C	Block D	All blocks		
Control	0	85.0	31.4	8.4	15.5	35.075	0	None
Chloropicrin	1	15.9	6.4	1.9	9.2	8.35[b]	76	$195
Chloropicrin	2	0.0	2.4	3.6	14.6	5.15[c]	85	$390
Chloropicrin-ethylene chloride 1: 9	10	0.0	0.0	0.0	0.0	0.00[c]	100[d]	$294
Chloropicrin-ethylene chloride 0.5: 9.5	10	2.8	0.9	0.0	0.0	0.925[c]	97[e]	$202
Ethylene chloride	10	0.0	0.0	0.0	0.0	0.00[c]	100[d]	$110
Methyl n. amyl ketone	10	12.5	52.9	6.1	34.9	26.60	24	Unknown
Chloropicrin-carbon tetrachloride 1: 9	10	12.4	5.4	0.0	0.0	4.45[c]	87	$300

[a] The injections in this experiment were all in rows 9 in. apart, holes 10.5 in. apart.

[b] Significantly different from control with odds of 19: 1. Minimum mean difference required for significance with odds of 19: 1, 21.58.

[c] Significantly different from control with odds of 99: 1. Minimum mean difference required for significance with odds of 99: 1, 29.37.

[d] Efficacy not less than 97 per cent with odds of 19: 1 as based on binomial distribution; 140 diseased bulbs in checks.

[e] Efficacy not less than 93 per cent with odds of 19: 1 as based on binomial distribution; 140 diseased bulbs in checks.

diseased in the control and the percentage diseased in the treatment by 35.075 and multiplying the result by 100.

Thus, a mean of 8.35 per cent for a treatment gives a difference of $35.075 - 8.35 = 26.725$ per cent. Therefore, the percentage efficacy is $26.725 \div 35.075 = .76$ or 76 per cent. This constitutes the percentage reduction in diseases.

Upon the basis of these results (Table 1), methyl n. amyl ketone may be eliminated. All other chemicals and dose rates were obviously beneficial. However, only those treatments containing ethylene chloride gave an efficacy either predictably or actually better than 90 per cent, and these are the only treatments considered recommendable on the basis of the experiment. Ethylene chloride in 10 cc. doses (15.3 cc. per sq. ft.) seems as good as or superior to all other treatments. This dose rate of ethylene chloride is the least expensive, costing only about $110 per acre, while chloropicrin, at 1 cc. per injection, costs about $195 per acre and is less effective for this particular nematode.

In another experiment (B), on *Ditylenchus dipsaci*-infested soil (injected to a depth of 6 in.), 6 randomized plots of each of 5 treatments were planted with 100 treated bulbs (4 hr. in 0.5 per cent Formalin at 110° F.) as previously described (Table 2). According to these results only the chloropicrin-ethylene chloride mixture (1:4) shows any promise. The percentage efficacy is lower than that of the various ethylene chloride mixtures or ethylene chloride alone in the previous experiment. Since less than half the quantity of ethylene chloride was used, this result might be expected. The efficacy of chloropicrin-ethylene chloride (1:4) is better than that of chloropicrin in 2 cc. doses in the previous experiment.

TABLE 2.—*Ditylenchus dipsaci in narcissus planted in chemically treated soil. Experiment B*[a]

Reagent	Cubic centimeters per injection	Percentage diseased bulbs	Mean	Percentage efficacy
Control	0	19, 100, 70, 49, 3, 17	43.00	0
Chloropicrin-ethylene chloride 1:4	5	15, 2, 5, 4, 2, 0	4.67[b]	89
Mesityl oxide	10	100, 23, 69, 7, 32, 28	43.17	0
Crotonaldehyde	10	70, 56, 45, 100, 37, 52	60.00	–40
Carbon disulphide	10	94, 57, 44, 35, 14, 76	53.33	–24

[a] The injections of chloropicrin-ethylene chloride were in rows 9 in. apart, holes 10.5 in. apart, while injections of the remaining treatments were in rows 6 in. and holes 7 in. apart.

[b] Significantly different from control with odds of 19:1. Minimum mean difference for significance with odds of 19:1, 33.35.

In both of these experiments with daffodil bulbs, flower and bulb weight records were kept but no differences were noted with the use of any chemical or combination of chemicals that could not be accounted for on the basis of reduction in percentage of diseased bulbs, except in 3 plots. In these 3 there was injury because chloropicrin was in the soil at planting time. No plant stimulation was detected.

EXPERIMENTS ON THE CONTROL OF ROOT KNOT NEMATODE

In still another experiment a greenhouse bed containing soil infested with the root-knot nematode, *Heterodera marioni,* and fungi, was divided into 12 plots and treated with chloropicrin and mixtures of chloropicrin and ethylene chloride. This bed had previously been planted to gerberas. The injections were made to a depth of 8 in. in rows 9 in. apart, holes 10.5 in. apart. Growth differences obtained are given in table 5. There were 2 plots for each soil treatment. In 9 of the plots 2 varieties and in 3 only 1 variety

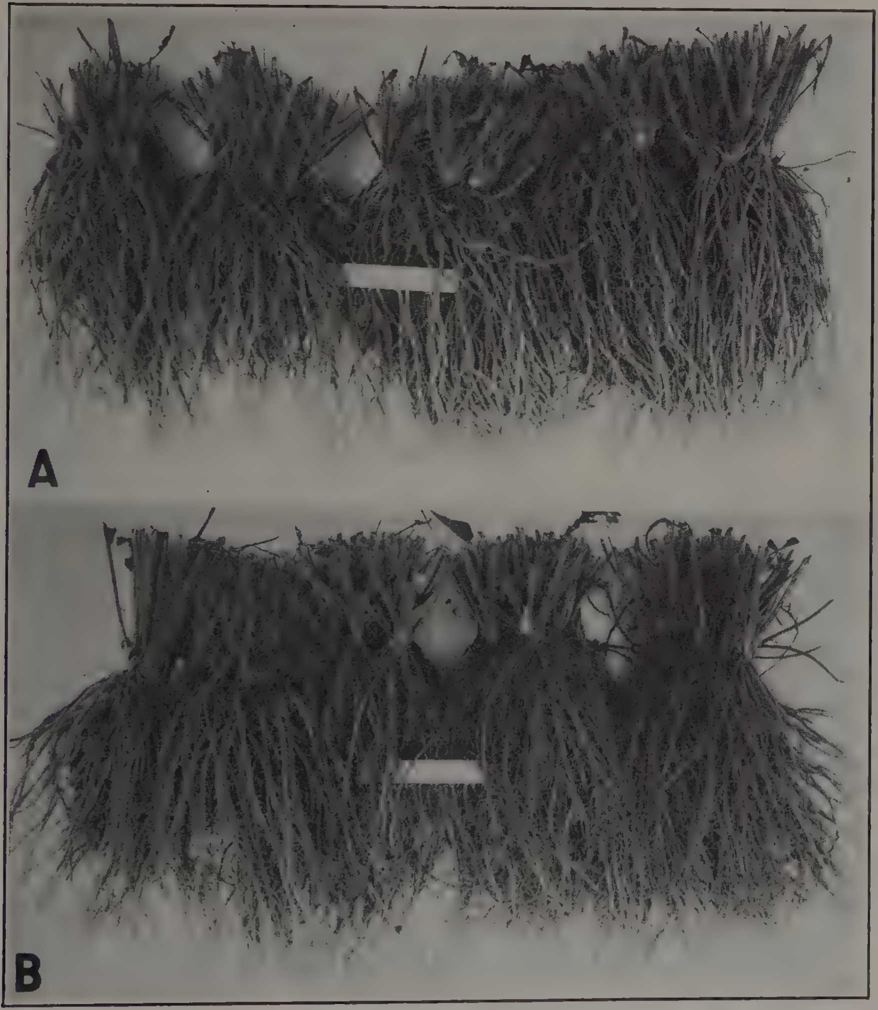

FIG. 1. Gerbera crowns and roots: A. Five samples selected at random from control plot. B. Five samples selected at random from plot treated with 10 cc. injections of chloropicrin-ethylene chloride 1.5: 8.5. Note development of secondary roots.

of gerberas were planted. Because of this fact no statistical analysis of the data has been made.

TABLE 3.—*Growth of gerberas in chemically treated soil*

Chemical	cc per injection	Mean height of flower stem in inches	
		Var. A	Var. B
Chloropicrin	0.5[a]	16.5	24.8
Chloropicrin	2.0	17.2	24.7
Chloropicrin-ethylene chloride 1.5: 8.5	10.0	18.5	27.6
	5.0	15.8	23.4
Chloropicrin-ethylene chloride 1: 4	5.0	16.2	23.9
Control	0.0	15.3	21.5

[a] Injector not working properly, dose rate probably higher.

From these results it would appear that mixture of chloropicrin and ethylene chloride 1.5: 8.5 in 10-cc. doses is the best. However, there is no proof that the benefit was not solely attributable to the ethylene chloride. Examination of the roots was made after a full year's growth. Since all of the gerbera plants were infected before planting, they were infected also at harvest. Gall counts were made on the roots, but they meant nothing, since the more vigorous plants had many times more secondary roots, which were gall-gearing, than did the poorer plants. However, the increase in secondary root growth was in complete accordance with the better flowering records. The plots treated with 10 cc. of chloropicrin-ethylene chloride 1.5: 8.5 were definitely superior (Fig. 1). The differences in root growth are probably due to reduction in varied plant diseases including both the root-knot nematode and fungi. This treatment is expensive, costing about $386 per acre, and it is not certain that the results could not be obtained at less cost. In this particular case, however, and possibly with other greenhouse crops, the increased crop value probably justifies the expense.

Finally, a report is made of an experiment on outdoor sandy loam, infested with the root-knot nematode. In this experiment 2 plots of each of 6 treatments, arranged in randomized blocks, were injected with chloropicrin, ethylene chloride, and mixtures of the two to a depth of 6 inches. Wet burlap bags were immediately applied thereafter to cover the soil. Each plot was finally planted with 14 seedling tomatoes, 2 inches tall, previously grown in clean sand. The mean stands of plants at the age of 6 weeks following the various treatments are given in table 4.

From the data on height of plants, it will be seen that the chloropicrin-ethylene chloride 1.5: 8.5 treatment worked best, and that the plain ethylene chloride treatment is more effective than the plain chloropicrin treatments and also less expensive.

The experiment was terminated because of a frost on October 3, after a growing period of 3 months. Few of the tomatoes had ripened, and the yields are probably considerably less than they would have been after a normal season's growth. The weight of all fruit was recorded on a plot basis. The mean crop weight per plant is given in table 4. These data indicate that the chloropicrin-ethylene chloride 1.5: 8.5 mixture is the best, and that all of the other treatments, except ethylene chloride, were significantly better than the control.

Examination also was made of the roots of each plant. It is difficult to give quantitative data as to infection with *Heterodera marioni.* All control plants were infected. In order to give some index of the degree of infection, even though the index be based on opinion, the following grade scale was set up: very severely diseased 5; severely diseased 4; moderately diseased 3; lightly infected 2; clean 0. The "root knot index" in table 4 is the total score of the plants divided by the number of plants. From this it will be seen that only one treatment, chloropicrin-ethylene chloride 1: 9 gave perfect results. However, both chloropicrin-ethylene chloride 1.5: 8.5 and ethylene chloride alone gave nearly complete control; only one plant bearing a light infection was found in each case. The differences are probably due to chance. The surprising lack of control with chloropicrin alone is difficult to explain. Only 9 plants in the 1 cc. chloropicrin plots and 6 in the 1.5 cc. chloropicrin plots escaped infection. The results are not in accordance with the yield records but are in reasonable accordance with

TABLE 4.—*The survival, growth, and yield of tomato plants grown in chemically treated soil previously infected with Heterodera marioni*[a]

Soil treatment	Dosage in cc. per injection	Number of plants surviving	Mean height, in inches, of living plants at 6 weeks	Yield in lbs. per plant	Root knot index	Cost per acre
Control	None	21	6.30	.40	3.83	None
Chloropicrin-ethylene chloride 1.5: 8.5	10	28	9.78[c]	1.06[c]	0.07[e]	$386
Chloropicrin-ethylene chloride 1: 9	10	27	9.37[b]	1.00[b]	0.00[d]	$294
Ethylene chloride	10	25	9.39[b]	.70	0.08[f]	$110
Chloropicrin	1.5	25	8.91[b]	.95[b]	3.10	$293
Chloropicrin	1.0	27	7.84	.79[b]	2.27	$195

[a] Differences in mean height required for significance with odds of 19: 1, 2.03 and odds of 99: 1, 3.18.

Differences in yield per plant required for significance with odds of 19: 1, .33 and with odds 99: 1, .52.

[b] Significantly better than control with odds of 19: 1.

[c] Significantly better than control with odds of 99: 1.

[d] No plants in 27 infected with root knot. Efficacy not less than 89 per cent, with odds of 19: 1 as based on binomial distribution with 28 out of 28 infected plants in control.

[e] One plant in 28 infected with root knot. Efficacy not less than 84 per cent, with odds of 19: 1 as based on binomial distribution with 28 out of 28 infected plants in control.

[f] One plant in 25 infected with root knot. Efficacy not less than 82 per cent, with odds of 19: 1 as based on binomial distribution with 28 out of 28 infected plants in control.

the records of height at 6 weeks. These results may be partly explained on the basis of results by Howard, Stark, and Smith (1939)[5] who found that partial sterilization may delay infection sufficiently in tomato plants to permit the formation of an adequate root system. Thereafter, attack by root knot may not cause much reduction in yield.

With root knot- and fungus-infested soil, it would appear that the selection of a chemical and dose rate would depend entirely on the value of the crop benefit.

SUMMARY

Chloropicrin, ethylene chloride, and mixtures of the two are effective in the control of nematodes in soil and consequently beneficial to plant growth. For narcissus eelworm, 10 cc. injections of ethylene chloride to a depth of 6 to 8 inches in rows 9 inches apart and holes 10.5 inches apart have been shown to be highly efficient. The cost is about $110 per acre. For soil for flats, the cost, including labor, would be about one-third cent per cubic foot. Other treatments also were efficient, but much more expensive.

For root-knot nematodes and fungus diseases of gerberas in greenhouse soil, a mixture of chloropicrin 1.5 cc. and ethylene chloride 8.5 cc. gave the best results. The cost is about $386 per acre.

For root knot and fungi of tomatoes, grown outdoors, the mixture of chloropicrin 1.5 cc. and ethylene chloride 8.5 cc. was the best from the standpoint of yield, but a 10 cc. injection of ethylene chloride was just as good or better from the standpoint of nematode control. Selection of a treatment should be based on the expected market value of the crop.

Caution should be exercised in the use of ethylene chloride for indoor work, since it is inflammable.

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[5] Phytopath. 29: 11. 1939.

THE GROWTH RATE OF SEVERAL WOOD-INHABITING FUNGI

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INTRODUCTION

The following experiments were planned to discover if possible what are the expressed effects on fungi of certain environmental factors. Six of the so-called higher fungi among the basidiomycetes, offering a variety of responses to the environmental stimuli thus considered, were chosen for these tests. In view of the effect of environment on vegetative growth of fungi, however, only a limited number of these factors was investigated. The factors considered, in the main involved light, temperature, moisture, hydrogen-ion concentration, and added enrichment of the agar substrate. It appeared evident from the beginning that of the factors under consideration the moisture content of the fungal substrate seemed to influence most the relative abundance of vegetative growth when the fungi were cultivated on solid agar media.

Fungi on improved agar media appeared to grow well at a pH range of 5 to 7. Somewhat better growth was obtained at the lower of the two pH determinations. An arbitrary standard pH of 6 was maintained throughout this study, however, because of the difficulty in maintaining the substrate solid at a lower pH when the percentage of agar in the media was lowered to 1 per cent.

REVIEW OF LITERATURE

Wilkins *et al.* (4) found that fungi, in nature, generally grew best at a pH range of 4–6. According to Wilkins *et al.* (4), this low pH range was true only of the humus substrate in which the mycelium proliferated and from which the sporophores arose. They found that the soil below this humus cover more often ranged in pH from 6–8 in the same habitat. To find the higher pH for the fungi under consideration is not surprising, since the fungi studied grew on wood in all cases and not on soil.

The effect of temperature on the growth and development of fungi is very marked in many instances. Cartwright and Findlay (1), in their studies on the physiology of wood-destroying fungi, in which rate of growth was observed in relation to temperature, noticed this marked difference in average daily increment of growth at different temperatures. In the main, the optimum average daily increments of growth for the wood-destroying fungi fluctuated between 23 and 27° C. They found the optimum average daily increments of growth for *Merulius lacrymans* to be 7.0 mm. at a temperature of 23° C., for *Pleurotus ostreatus* a daily increment of 17.0 mm. at 25° C., and for *Lentinus lepideus* an average daily increment of 9.9 mm. at a temperature of 27° C.

The lower the concentration of agar in the media the more prolific appeared to be the growth of the mycelium of the various fungi. To maintain a solid consistency to the agar substrate, the concentration was maintained throughout the experiments at a maximum of 1 per cent. Groom and Panisset (5), studying the growth of fungi on library books, found that the relative humidity of the air at which mildews developed varied inversely with the temperature, and that at a temperature of 25° C. the humidity of the air for optimum growth was 72.6 per cent.

Choice of media in the growth and development of fungi is evidently important. Hall (2) found for *Sclerotinia fructigena* meager growth on Richard's agar and sparse growth on prune agar. He found that there was a large development of mycelium on potato-mush agar, as well as on the synthetic media developed by Brown.

Not all environmental factors seem to function equally well in limiting fungous growth and development. According to some authors, light and humidity appear not to affect growth and development very markedly. On the other hand, pH, temperature, and nutrients seem to exert a marked effect on fungous growth. Heat at suitable temperatures allows for abundant growth, while at unfavorable temperatures the growth of the fungus is reduced enormously.

MATERIALS AND METHODS

Of the 6 fungi used in these experiments all, except the single polypore, *Merulius lacrymans* (Wulf), belong to the division of basidiomycetes called the mushrooms. The mushrooms used were *Collybia velutipes* Fr., *Pleurotus ostreatus* Fr., *P. ulmarius* Fr., *Pholiota adiposa* Fr., and *Armillaria mellea* Fr. Of the six fungi used, *C. velutipes* and *Pleurotus ostreatus* were distinctly rapid growers, while *A. mellea* proved to be a decidedly slow grower.

The fungi were cultured in Petri dishes 9.2 cm. in diameter. The amount of sterile medium aseptically poured into each Petri plate was measured exactly to 15 cc. All tests were set up in triplicate series and the experiments were repeated twice to secure comparable data. This procedure was consistently followed in making the study of all 6 fungi.

During the period of growth the cultures of fungi were kept in an incubator at a uniform temperature of 25° C. After the plantings were made, measurements were taken at 48-hr. intervals and continued regularly until the fungous mat had completely covered the surface of the agar. Each culture was started by the transfer of a 3-mm. block of agar taken from a plate in which the fungus had been actively growing on the test media. The transplants were placed with the aerial mycelium uppermost. Each fungus was started originally from a hyphal tip and, prior to its growth in the final tests, the fungi were cultured for 3 successive plantings on the identical media in which the test cultures were finally grown. All the fungi, therefore, were equally well acclimatized to the media on which they grew before the start of the test experiments.

Preliminary tests in hydrogen-ion concentration covering a pH range from 5 to 8 were made at pH intervals of .5. The lower pH values appeared definitely good for fungus growth. However, a pH value of 6 was finally arbitrarily established in order to minimize the difficulties encountered in maintaining a solid medium at a lower pH value when the agar concentration was limited to 1 per cent.

Likewise, wide ranges of temperature were initially investigated, and, eventually, narrower ranges were tested to determine an optimum temperature. The 5 temperatures of the preliminary tests ranged from 15 to 35° C. with intervals of 5°. This wide range was at length narrowed down to the temperatures of 23, 25, and 27° C. from which finally the 25° C. temperature was selected as the most satisfactory for the equitable growth of the fungi under investigation.

A similar approach was adopted in determining the most favorable concentration of agar. Ranges such as 1, 2, and 3 per cent concentration of agar were used at first; then a narrower and lower range of .5, 1, and 1.5 per cent concentrations was investigated. Some difficulty was experienced in handling an agar medium of less than 1 per cent, so that percentage concentration was used throughout the experiments and proved very satisfactory.

The rate of growth of the fungi under consideration was further observed under 3 degrees of light intensity; namely, full daylight, shade, and complete darkness. The rate of growth, measured diametrically, was not greatly decreased in full daylight, nor appreciably increased in darkness. On the contrary, the surface appearance of the mycelial mat was quite marked. Fruiting and distinct concentric rings were evidenced in full daylight, while an elevated and more uniformly even growth was evidenced in complete darkness. The shade exposure gave the greatest diametrical growth. The data recorded in these experiments were taken from the fungus series cultured in the shade, and the photographic plates submitted, likewise, were made from this light exposure.

The regular basic ingredients were added to all media; namely, sugar (sucrose 2 per cent), potato extract (200 g. per l.), salt (sodium chloride 0.5 per cent), peptone (Bacto 1 per cent), and agar (Bacto 1 per cent).

The addition of several liquor extracts was tried as an enrichment to the basic media. These extracts were secured from sources such as corn, pea, carrot, and prune. The liquor extract from prune gave, on the whole, the best growth results. In all the tests recorded here, the basic media were enriched by adding to each liter of agar the juice from ½ lb. of steamed prunes. The enriched media were used in the tests for making relative growth comparisons of all 6 of the selected fungi.

The factors chosen for study in this piece of research are by no means complete, nor are the methods and procedures followed in making the determinations in any way ideal, but in so far as the selection extends, the factors have been very carefully developed and the procedures checked.

An attempt at evaluation of growth factors and growth responses of fungi in the field may be profitably undertaken in order to learn more about the little-known ecology of the higher fungi.

DATA AND DISCUSSION

The first series of fungi was planted in triplicate cultures on March 31, and measurements of the diametric growth on an agar surface were taken every other day over a period of 1 month. The average of 3 readings was recorded in all cases. The results of the measurements are recorded in table 1. In approximately 11 days the mycelial mats of the fungi *Collybia velutipes* and *Pleurotus ostreatus,* 2 wood-inhabiting mushrooms, covered the agar surface in the Petri plates, a distance of 9.2 cm., giving a growth rate of practically .8 cm. every 24 hours.

TABLE 1.—*Daily record of growth in diameter, measured in centimeters, and average daily increment of growth of six species of wood-inhabiting fungi*

Date	*Collybia velutipes*	*Pleurotus ostreatus*	*Merulius lacrymans*	*Pleurotus ulmarius*	*Pholiota adiposa*	*Armillaria mellea*
March 31	Planted	....	....	....	....	....
april 2~~1~~	0.70	0.45	0.65	0.35	0.40	0.35
" 5	3.65	3.50	1.70	1.40	1.35	0.60
" 7	5.30	5.25	2.65	2.60	1.70	0.75
" 9	6.40	6.65	3.40	3.85	2.30	1.00
" 12	9.10	9.20	6.15	6.25	4.20	1.50
" 15	Covered	Covered	8.15	6.90	5.15	1.70
" 17	....	....	9.10	8.40	6.45	2.20
" 20	....	....	Covered	9.15	7.15	2.65
" 22	....	....	....	Covered	7.50	2.85
" 25	....	....	....	....	8.05	3.10
" 27	....	....	....	....	8.45	3.50
" 29	....	....	....	....	8.80	3.75
May 2	....	....	....	....	Covered	3.90
Averages	0.76	0.77	0.54	0.46	0.30	0.12

Two wood-destroying fungi *Merulius lacrymans* and *Pleurotus ulmarius,* covered the surface of the agar with their mycelial mats in 19 days, thus growing at the rate of approximately .5 cm. every 24 hours.

The tree-inhabiting mushroom, *Pholiota adiposa,* completely covered the surface of the agar with its mycelial mat after the lapse of 30 days, growing at the slow rate of only .3 cm. every 24 hours. On the other hand, *Armillaria mellea,* a root-destroying fungus, had developed through a diameter of 3.9 cm. in the longest period of time the experiment was in progress, namely 32 days. The growth rate in this instance was only .12 cm. every 24 hours. *Armillaria mellea* thus proved to be an exceedingly slow grower on an artificial medium.

The data recorded in table 2 were obtained from a second series of fungus cultures planted on April 19 and completed on May 21. The records made in this instance were also the average of 3 readings, and, in the second series of cultures, the measurements were checked by an efficient laboratory

TABLE 2.—*Daily record of growth in diameter measured in centimeters and average daily increment of growth of six species of wood-inhabiting fungi*

Date	*Collybia velutipes*	*Pleurotus ostreatus*	*Merulius lacrymans*	*Pleurotus ulmarius*	*Pholiota adiposa*	*Armillaria mellea*
April 19	Planted	....	....	....	....	....
" 21	0.70	0.45	0.65	0.35	0.40	0.35
" 23	1.70	1.40	1.75	1.40	0.85	0.45
" 26	4.85	4.70	3.35	2.75	1.60	0.90
" 28	7.90	7.70	4.05	4.50	2.60	1.35
" 30	9.15	9.05	5.10	5.75	3.25	1.65
May 3	Covered	Covered	7.65	6.85	4.65	1.80
" 5	....	....	8.90	8.05	5.59	2.15
" 7	....	....	Covered	9.15	6.65	2.50
" 10	....	....	....	Covered	7.45	2.75
" 12	....	....	....	....	8.00	3.00
" 14	....	....	....	....	8.50	3.25
" 17	....	....	....	....	8.90	3.65
" 19	....	....	....	....	Covered	3.90
" 21	....	....	....	....	....	4.25
Averages	0.83	0.82	0.56	0.51	0.32	0.13

worker. The period of time taken by each fungus to span the distance from the center of the Petri plate to its periphery was in all cases comparable to that elapsing in the first series of cultures to cover the same distance, namely that recorded in table 1.

The average daily increment of growth for each fungus, calculated from the data recorded in tables 1 and 2, is 8 mm. for *Collybia velutipes,* 8 mm.

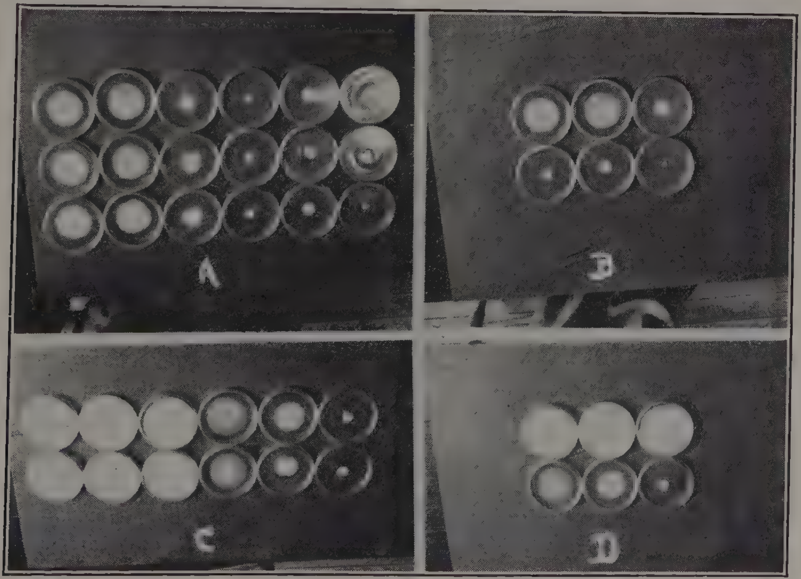

FIG. 1. Plate cultures, A (left to right), of *Collybia velutipes, Pleurotus ostreatus, Merulius lacrymans, Pleurotus ulmarius, Pholiota adiposa,* and *Armillaria mellea* 8 days after the fungi were planted. B. One culture each of all 6 fungi arranged in the order designated for A. Figures C and D show the same fungi in the same order just 15 days after planting.

for *Pleurotus ostreatus,* 5.5 mm. for *Merulius lacrymans,* 4.8 mm. for *P. ulmarius,* 3 mm. for *Pholiota adiposa* and 1.2 mm. for *Armillaria mellea.*

Cultures shown in figure 1 indicate a close similarity in rate of growth of the two mushrooms *Collybia velutipes* and *Pleurotus ostreatus.* There is also a fairly close similarity in rate of growth between the two fungi *Merulius lacrymans* and *Pleurotus ulmarius,* even though for the latter two fungi the rate of growth is much slower than for the two former ones. The rate of growth of *Pholiota adiposa* is slightly more than one-third that of *Collybia velutipes* and that for *Armillaria mellea* is exceedingly slow, amounting to only 1.2 mm. increment of growth every 24 hours. *Collybia velutipes,* on the other hand, makes an increment of growth equivalent to 8 mm. every 24 hours under the same restricted growing conditions.

Data recorded in tables 1 and 2 indicate rather clearly that there are very great differences in the rates of growth among closely related fungi, even when all environmental factors are kept uniform. Furthermore, the rate of growth for any individual fungus can be duplicated repeatedly so long as the environmental factors are maintained constant and uniform, as originally established for experimental purposes.

The differences in rates of growth seem to be due to some or all of such factors as the environmental conditions surrounding the fungi, the constituents or components of the substrate on which the fungi grow, and the natural inheritable capacities of the fungi. The latter two of these factors appear to be the more probable determining ones; especially, since the surrounding environmental conditions, such as light, temperature and moisture, are in most cases constant factors of an environment.

SUMMARY

Six fungi were selected for these studies, namely *Collybia velutipes, Pleurotus ostreatus, Merulius lacrymans, Pleurotus ulmarius, Pholiota adiposa* and *Armillaria mellea.*

The fungi were cultivated upon fruit-enriched agar, in Petri dishes, having a uniform depth and a diameter of 9.2 centimeters.

Uniform conditions of temperature, hydrogen-ion concentration, light intensity, moisture, and substrate components were maintained.

The fungi were all cultivated in the same month of the year and grown for the same period of time.

Different rates of growth were found for these fungi, although they were cultivated under equally uniform conditions.

All the factors for growth were maintained as far as possible at optimum conditions or close to optimum and sufficient controls were handled to compute a satisfactory mean measurement.

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LITERATURE CITED

1. CARTWRIGHT, K. G., and W. P. K. FINDLAY. Studies in the physiology of wood-destroying fungi. Ann. of Bot. **48**: 481–496. 1934.
2. HALL, MURIEL P. An analysis of the factors controlling the growth form of certain fungi, with especial reference to *Sclerotinia fructigena*. Ann. of Bot. **47**: 543–578. 1933.
3. BARNES, B. Variations in *Botrytis cinerea* induced by the action of high temperature. Ann. of Bot. **44**: 825–858. 1930.
4. WILKINS, W. H., J. L. HARLEY and G. C. KENT. Ecology of the larger fungi. (b) Distribution in relation to type of substrate. (c) Hydrogen-ion concentration. Ann. App. Biology **25**: 472–489. 1938. Also, **24**: 703–732. 1937.
5. GROOM, PERCY, and THÉRÈSE PANISSET. Studies on *Penicillium chrysogonum* in relation to temperature and relative humidity of air. Ann. App. Biology **20**: 633–660. 1933.
6. LINDGREN, R. M. Decay of wood and growth of some hymenomycetes as affected by temperature. Phytopath. **23**: 73–81. 1933.

THE RELATION OF THE CURCULIONID, ANACENTRINUS DEPLANATUS, TO ROOT ROT AND BASAL STEM ROT OF BARNYARD GRASS, ECHINOCHLOA CRUSGALLI[1]

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INTRODUCTION

Insect attacks on the lower parts of the plants may be one of the most important factors influencing the development of root rot and basal stem rot of cereals and grasses. Heavy insect infestation at the bases of these plants is common in the Northern Great Plains and severe basal stem rot is almost invariably associated with such insect injury. Several kinds of insects are involved, but certain species of weevils are particularly important. They commonly infest the crowns and bases of stems of wheat, barley, rye, and many wild and cultivated grasses. Their entrance weakens the plants, provides avenues of entrance for fungi and bacteria, and sometimes fills the culms with frass, a good medium for the rapid growth of microorganisms. Hanson and Christensen (2) recently called attention to the problem and listed a number of widely distributed grasses that are attacked by these insects. One of the hosts listed, *Echinochloa crusgalli* (L.) Beauv., popularly known as barnyard grass, is frequently attacked by the weevil, *Anacentrinus deplanatus* (Casey). Barnyard grass remains green until late in the season and apparently is an ideal host for this weevil.

SYMPTOMS

Infestation varies with the season and locality. Extent of host injury depends on the time of year that infestation occurs and the subsequent environmental conditions. When plants are attacked early, and dry weather ensues, they may become dwarfed and most of the basal leaf sheaths and some of the culms will be killed. When infested late in the season or under conditions exceptionally favorable to growth of the host, there may be no marked differences in the appearance of infested and noninfested plants. Infestation may occur sometimes before and any time after they begin heading. Brown discoloration often appears on the basal parts of the culms of infested plants as a result of outward decay from within or the development of decay around the oviposition punctures (Fig. 1). It is not always possible to detect infested plants by external symptoms. Sectioning of the culms may, therefore, be necessary to reveal the discolored and diseased areas (Fig. 2). The affected part may be limited to the proximity of the crown or may extend upward for several inches. As a rule, the insect tunnels are filled with frass, which usually becomes discolored and necrotic along with the culm.

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[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Divisions of Plant Pathology and Botany, and Entomology and Economic Zoology of the Minnesota Agricultural Experiment Station. Paper No. 1848 of the Scientific Journal Series of the Minnesota Experiment Station.

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[2] The writers gratefully acknowledge their indebtedness to Dr. J. J. Christensen for suggesting this problem and for much valuable criticism and help during the progress of the investigation. Thanks are due also to Drs. W. A. Riley, Helen Hart, C. M. Christensen, and C. J. Eide for reading and criticizing the manuscript.

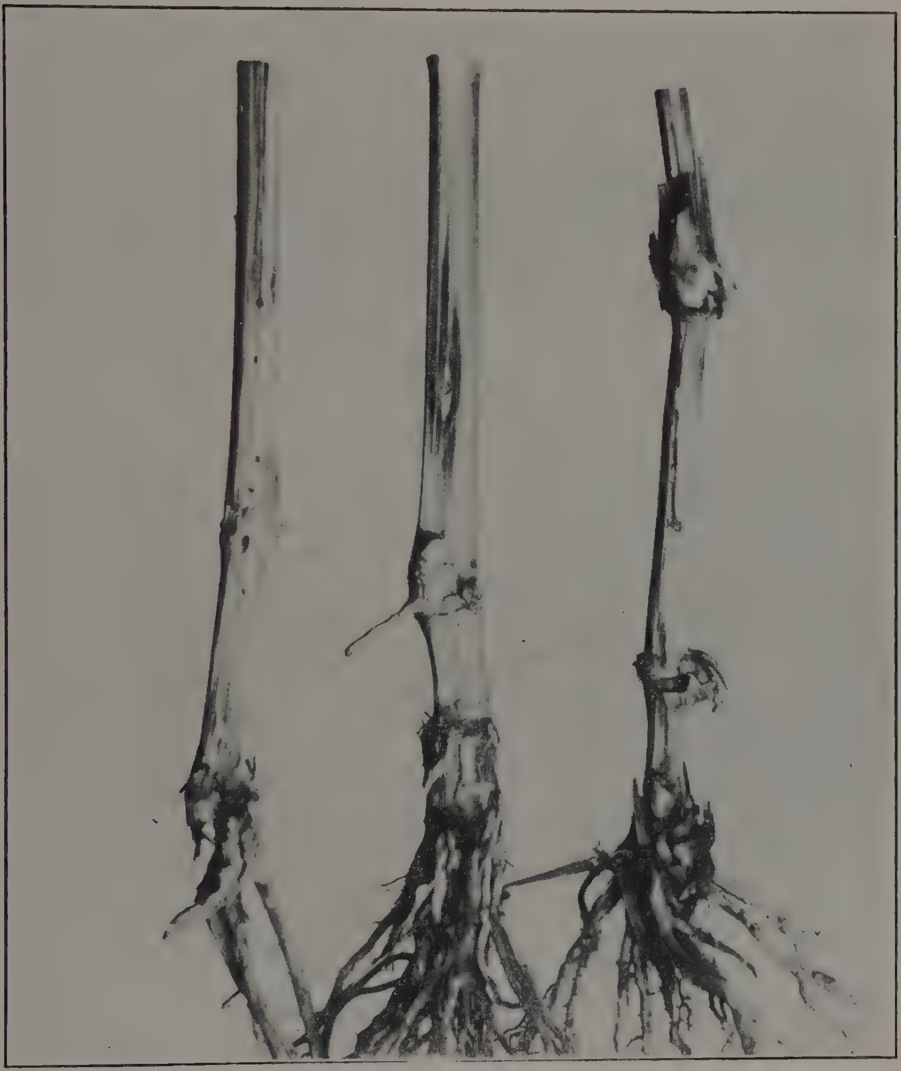

FIG. 1. Bases of culms of *Echinochloa crusgalli*, showing external symptoms of infestation and decay. The middle culm shows evidence of rot-inducing organisms that entered through the oviposition punctures. Approximately × 1.

DESCRIPTION OF INSECT

Anacentrinus deplanatus (Casey) (= *Limnobaris deplanata* Casey) is the weevil most commonly associated with the basal stem rot of barnyard grass. Although the life history of this insect has not been completely ascertained for the vicinity of St. Paul, it is known that infestation occurs from June to at least the middle of September. During this period, larvae may be found anywhere in the culms of infested plants from the root crown to a distance of 2 to 3½ inches above the soil line. Usually there is but one larva in a culm or crown, but 2 are not uncommon and sometimes as many as 3 are found. Early in the season the larval stage is most prevalent, while in the fall the pupal or adult stages predominate. All observed cases of pupation occurred in hollowed chambers within the plant (Fig. 2). The full-grown larva (Fig. 3, A) measures approximately 2.5 × 1.3 mm., in a natural, curved position, and is white, except for the amber head. The pupa (Fig. 3, B and C)

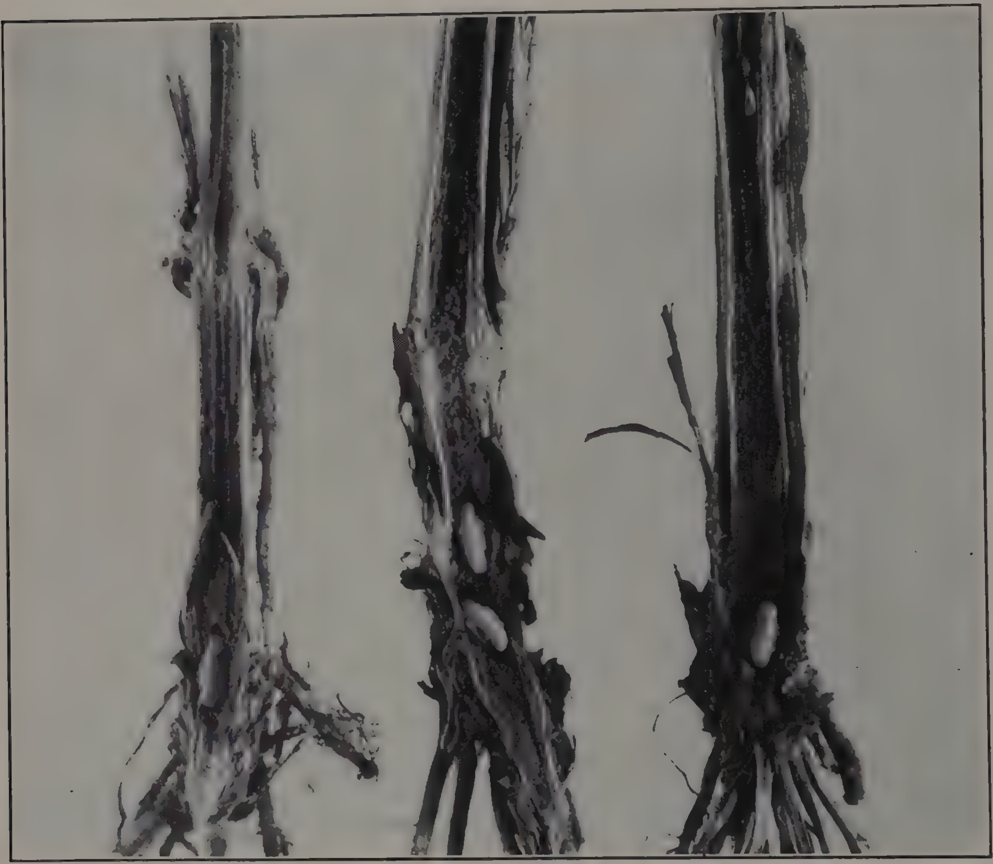

FIG. 2. Longitudinal sections of infested stem bases of *Echinochloa crusgalli*, showing extent and nature of infestation and the pupae of *Anacentrinus deplanatus* in their respective pupal chambers. Approximately × 3.

measures 2.5 × 1.6 mm.; during the first few days it is entirely white, but becomes a dull brown prior to emergence of the adult (Fig. 3, D and E). The adult is 3.0 mm. long and 1.2 mm. wide. Newly emerged individuals are pale to reddish brown, while older specimens are black, except for the minute, white setae-like scales.

This species has been definitely recorded by Buchanan (1) from Arkansas, Illinois, Kansas, Louisiana, Mississippi, Missouri, South Dakota, Texas, and Washington, D. C. Barnyard grass appears to have been the only host mentioned prior to the publication by Hinds and Osterberger (4), who found *Anacentrinus deplanatus* associated with a closely related species, *A. subnudus* Buch., causing considerable damage to sugar-cane plantings in Louisiana. Earlier, in 1930, Ingram and Holloway (5) discussed the damage done to sugar-cane rootstocks and young plants by weevils, which were, according to Buchanan (1), *A. subnudus*. They state that "in some cases the stubble was riddled with tunnels and all eyes killed either directly by the weevil larvae or by the entrance of disease organisms into the tunnels."

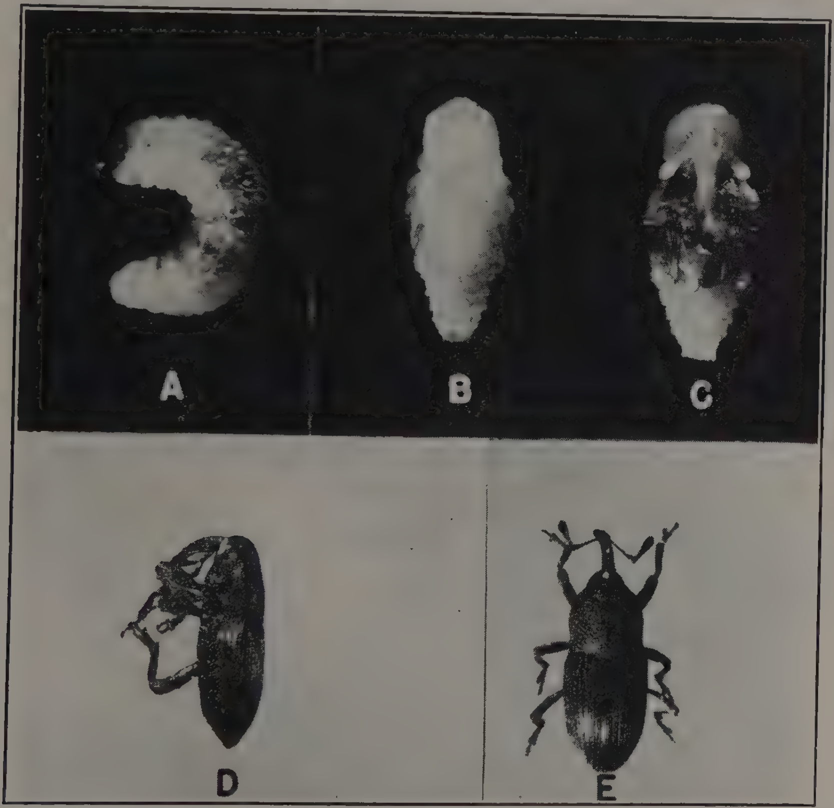

FIG. 3. Stages in the life history of *Anacentrinus deplanatus*. A. Larva. B. Pupa (dorsal view). C. Pupa (ventral view). D. Adult (lateral view). E. Adult (dorsal view). ×9.5.

INSECT INFESTATION IN RELATION TO DEVELOPMENT OF ROTS

In the research here reported, many kinds of soil fungi and bacteria were found associated with root rot and basal stem rot of barnyard grass, and the amount and severity of the decay was consistently greater in the weevil-infested than in the noninfested plants. The weevil not only wounds the plant, thus weakening it and furnishing invasion avenues for the fungi and bacteria, but it also deposits a frass in its tunnels, which greatly facilitates the rapid growth and multiplication of these organisms. Some fungi, particularly certain species of *Penicillium,* sporulate abundantly in these tunnels. Moreover, once the organisms have become established they can spread beyond the insect tunnels into the adjacent noninfested tissue.

Approximately 500 infested plants and an equal number of noninfested ones were collected at random from several fields, surface-sterilized with mercuric chloride, and plate cultures were made (Table 1).

TABLE 1.—*Percentage of infection and relative prevalence of various fungi and bacteria associated with the decay of the basal parts of insect-infested and noninfested plants of Echinochloa crusgalli*

Source and part of plant	Total infection	Relative percentage prevalence of fungi and bacteria isolated from infected plants[a]						
		Fusarium	Helminthosporium	Alternaria	Mucors	Trichoderma	Other fungi	Bacteria
	Per cent							
Infested plants								
Basal parts of stems	95	69	7	1	0	10	2	11
Roots	68	41	43	5	0	4	1	6
Frass	100	50	1	1	19	16	2	11
Noninfested plants								
Basal parts of stems	15	50	36	4	0	0	5	5
Roots	49	48	33	5	0	0	6	8

[a] Percentages given in nearest whole numbers.

The percentage of infection was strikingly greater on the insect-infested than on the noninfested plants, even though root rot was common on the latter. The frass in the insect tunnels was always infected. Species of *Fusarium,* including both *F. culmorum* (W. G. Sm.) Sacc. and *F. graminearum* Schw., were isolated with the greatest frequency. Several species of *Helminthosporium,* especially *H. sativum* P. K. and B., were very prevalent during the early part of the summer but by the first week of September, when the major part of the plating was done, they constituted only 7 per cent of the isolates from infested stems. The relative prevalence of *Helminthosporium* on the stems was considerably higher on noninfested than on infested plants. These data confirm the observations of the senior writer on other foot and root rots of cereals and grasses, *i.e.,* that the prevalence of *Helminthosporium* relative to other genera of fungi is higher on young and vigorously growing plants than on those approaching maturity, or those greatly weakened by disease, insects, or other unfavorable conditions. Less pathogenic organisms seem to be able to compete with *Helminthosporium* better when, for any reason, the growth of the plants is retarded. Some of the other fungi commonly isolated were: *Alternaria* spp., *Aspergillus* spp., *Basisporium* spp., *Brachysporium* spp., *Cephalothecium* spp., *Chaetomium* spp., *Penicillium* spp., *Rhizoctonia solani* Kühn, and *Stemphylium* spp. At least 3 kinds of bacteria were isolated consistently from the infected areas of the plants. Larvae, pupae, and adults were found to have all of these fungi and bacteria on the exterior parts of their bodies. Also, when they were surface-sterilized and plated out, they were found to contain several species of *Fusarium,* including both *F. culmorum* and *F. graminearum; H. sativum, H. pedicellatum* Henry (3), and several species with narrow and short conidia; *Trichoderma* sp.; *Alternaria* sp.; *Penicillium* sp.; *Rhizoctonia solani* Kühn; and several genera, which were not identified. The same types of bacteria isolated from infested plants were commonly isolated from the body contents of the larvae.

Although *Anacentrinus deplanatus* has been definitely reported only from barnyard grass and sugar cane, it seems probable, or at least possible, that it attacks other hosts. Similar larvae have been noted in barley and several grasses. The weevil causing most of the damage to wheat is a different species, but occasionally larvae are found that resemble those of *A. deplanatus.* It will be necessary to make a separate study for each host before the species of weevil concerned in each case will be known. All of the weevils attacking the basal stems of cereals and grasses in the Northern Great Plains area cause similar damage, and it is evident that they increase the severity of both root rot and basal stem rot. The development of varieties resistant to these diseases must take into consideration their susceptibility or resistance to the insects that predispose them to attack, as well as the inter-relationships between these insects and the rots.

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LITERATURE CITED

1. BUCHANAN, L. L. A new barine curculionid injurious to sugar cane in Louisiana with synopses of *Anacentrinus* and *Oligolochus* (Coleop.). Ann. Ent. Soc. Amer. **25**: 328–336. 1932.
2. HANSON, E. W., and J. J. CHRISTENSEN. Insects in relation to root rot and basal stem rot of cereals. (Abstract) Phytopath. **30**: 7–8. 1940.
3. HENRY, A. W. Root-rots of wheat. Minn. Agr. Exp. Stat. Tech. Bull. 22. 1924.
4. HINDS, W. E., and B. A. OSTERBERGER. Sugar cane rootstock weevils. Ann. Ent. Soc. Amer. **25**: 337–343. 1932.
5. INGRAM, J. W., and T. E. HOLLOWAY. Injury to sugar cane by a small weevil. Jour. Econ. Ent. **23**: 832–833. 1930.

CURRENT-SEASON DEVELOPMENT OF VIRUS SYMPTOMS IN TULIPS

PHILIP BRIERLEY

(Accepted for publication January 7, 1941)

In recent years leaf rubbing with the aid of carborundum powder has gained wide acceptance as an effective method of inoculation for many plant viruses. This paper presents the first evidence that this method is satisfactory for viruses transmissible to tulip and will yield current-season symptoms when used at an early stage of plant growth. This is in strong contrast with the earlier published experiences of the writer and coworkers (2, 3, 4, 11, 12). McWhorter, in a private communication in 1940, reported his first observed current-season effects as also following early-season inoculation by leaf rubbing. Apparently, inoculation at an early stage of development of the tulip is the chief requirement for current-season symptom expression. The leaf-rubbing method lends itself to early-season inoculations and is simple, fast, and effective.

Cayley (5, 6) grafted bulbs during the storage period and inserted cores of tissue from diseased bulbs into healthy bulbs. Gigante (7) used these and similar methods of inoculation into bulbs. He also made use of capillary tubes to inject sap expressed from leaves of "broken" tulips into the leaves of healthy tulips shortly after the latter appeared aboveground. All these methods yielded current-season effects, in low proportion in Cayley's experiments, in high proportion but accompanied by weak growth and blindness in Gigante's trials. McKenny Hughes (8, 9, 10) consistently obtained current-season breaking symptoms in trials with vector species of aphids. In one of his experiments *Myzus persicae* produced current-season breaking in 85 per cent of the tulips infested. McKenny Hughes' uniform success in producing breaking in the same year, contrasting with our (4) failure to produce any current-season breaking with the same vector species, may be attributed to his practice of preparing viruliferous colonies suitable for transfer very early in the season.

In preliminary trials in 1938 the "strong-mottle" virus of Easter lily was inoculated from this species into *Lilium formosanum* by leaf rubbing with carborundum, by multiple needle puncture, and by hypodermic needle injection (Luer syringe, No. 27 needle). The numbers of plants inoculated were 10 in each instance and infections were 9, 4, and none respectively. In a parallel trial with inoculum from necrotic-flecked Easter lily, 8, 1, and 2 plants of *L. formosanum* were infected. Leaf rubbing was adopted as the standard method of inoculating lily viruses into plants other than tulip.

The hypodermic-needle method was retained for inoculations into tulips largely because of the effectiveness of transfer reported by McWhorter (11). This method seemed to offer more promise for these plants because tulip peduncles are readily injected by needle with considerable quantities of liquid. When Clara Butt tulips growing in an unprotected outdoor frame were inoculated with viruses from Easter lily by hypodermic needle in 1937, 103 of 167 plants (61.7 per cent) showed infection in 1938. When this tulip variety was inoculated under similar conditions in 1938, 12 of 37 plants (32.4 per cent) inoculated with viruses of the tulip type, and 6 of 18 inoculated with cucumber virus 1 strains showed infection in 1939. These proportions of transfer are lower than those reported by McWhorter (11) for the hypodermic method under field conditions in Oregon.

Paired lots of Clara Butt tulips were inoculated in the greenhouse on March 3 and 4, 1939, one with the hypodermic needle and the other by leaf rubbing with carborundum. Tulip viruses 1 and 2, the latent virus of lily, the strong-mottle virus of Easter lily, and several strains of cucumber virus were included in these comparisons. With each virus the same sample of expressed juice, diluted approximately 1:20 with tap water, served as inoculum for both methods. The inoculations were delayed until flower buds were beginning to show color in order to permit injection of flower stems by the needle method. No symptoms appeared during the current season. All bulbs produced by the inoculated plants were grown in the open for record in 1940. Infection was expressed chiefly as flower symptoms, but the cucumber-virus strains, lily latent virus, and strong-mottle virus also produced well-defined leaf symptoms. The data in table 1 show leaf rubbing to be superior to hypodermic-needle injection for infection with these viruses under the conditions of the comparison. Cucumber viruses produced 16 infections in 101 plants (15.8 per cent) following hypodermic inoculation, and 30 in 94 plants (31.9 per cent) following leaf rubbing. The tulip viruses of McWhorter (11) and the latent and strong-mottle viruses of lily, tentatively grouped with these (2, 12), caused 17 infections in 55 plants (30.9 per cent) by hypodermic injection, and 29 in 49 plants (59.2 per cent) by leaf rubbing.

TABLE 1.—*Comparative results of hypodermic-needle vs. leaf-rubbing inoculation of viruses into Clara Butt tulips. Inoculations March 3 and 4, 1939; symptoms recorded May 14, 1940*

Virus	Source	Hypodermic	Leaf-rubbing
Tulip virus 1	Tulip	1/11[a]	2/6[a]
Tulip virus 2	do.	11/16	13/17
Latent virus of lily	Easter lily	1/13	3/15
Strong-mottle virus of Easter lily	do.	4/15	11/11
Cucumber virus 1, lily strains	do.	5/51	10/45
do.	Tobacco	11/50	20/49
Controls, uninoculated		0/27	

[a] Number of plants infected over number surviving.

In 1940, Clara Butt tulips, forced in flats in a greenhouse, were inoculated by the leaf-rubbing (carborundum) method with a number of virus collections from lily and with the two tulip viruses distinguished by McWhorter (11). On January 19, when the first inoculations were made, each tulip shoot was still enclosed within the outermost leaf and the outer surface of this was rubbed. Later inoculations, in February, were applied to this leaf, to the second leaf, and in some plants to the third leaf also. Many of these inoculations produced current-season flower breaking and several cucumber-virus strains also produced leaf symptoms (Table 2). The cucumber-virus strains infected 46 out of 110 plants (41.8 per cent); tulip viruses, 101 out of 210 plants (48.1 per cent) and the inoculations from lilies carrying virus complexes were successful in 53 out of 120 plants

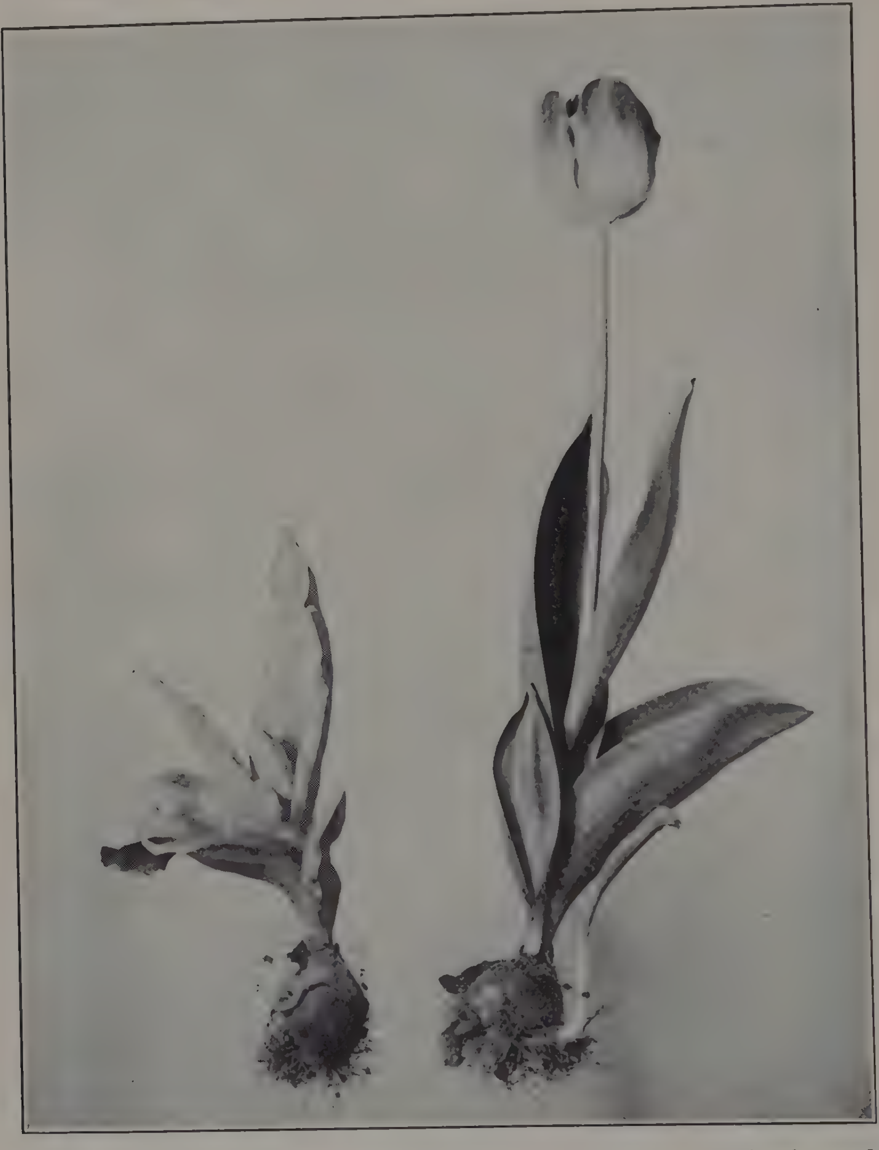

FIG. 1.—Symptoms produced in Clara Butt tulip by a virulent lily strain of cucumber virus. 1. Photographed March 12, 1940, 53 days after inoculation. Healthy Clara Butt at the right.

TABLE 2.—*Production of current-season virus symptoms in Clara Butt tulips by leaf-rubbing inoculation with carborundum. Records in March and April, 1940*

Date of inoculations (1940)	Virus type	Source plant	Number of plants			Leaf symptoms
			Inoculated	Bloomed	Infected	
Jan. 19	Cucumber virus 1 (lily strains)	Tobacco	10	10	8	+
Feb. 10	do. do.	do.	20	2	7	+
Feb. 12	do. do.	*L. longiflorum*	60	13	16	+
Feb. 12	do. do.	*L. formosanum*	20	13	15	+
Feb. 15	Tulip virus 1 (McWhorter)	*L. longiflorum*	10	8	8	–
Feb. 15	do. 2 do.	do.	10	7	1	–
Jan. 19	"Strong-mottle" virus	do.	10	9	2	–
Feb. 12	do.	do.	20	12	11	–
Feb. 15	"Latent virus of lily"	do.	30	19	18	–
Feb. 15	do.	*L. formosanum*	80	36	33	–
Feb. 16	do.	*L. longiflorum*	20	16	12	–
Feb. 16	do.	*L. candidum*	20	15	15	–
Feb. 16	do.	*L. speciosum*	10	10	1	–
Feb. 8	"Necrotic-fleck" complex	*L. longiflorum*	30	17	8	–
Feb. 8	"Yellow-top" complex	do.	30	27	25	+
Feb. 8	"Chlorotic-fleck" complex	do.	30	18	11	–
Feb. 8	"Very-sparse-fleck" complex	do.	30	8	9	+

Controls: 60 uninoculated plants; 57 bloomed; all normal.

(44.2 per cent). Inasmuch as cucumber viruses produced recognizable leaf symptoms but tulip viruses did not, diagnosis of the latter was dependent on flowering of the tulips. The number of flowers was low in this series because the tulips were a little too dry when forced. There is reason to expect that early inoculation by leaf rubbing may prove more effective if the tulips are well-grown.

All cucumber-virus strains tested produced similar current-season symptoms in tulips. The flower breaking characteristic of cucumber virus is a dull-red striping or feathering. These lines of intensified color are less sharply demarked and duller in tone than the corresponding effects of tulip viruses. Commonly, there is a conspicuous blemish near the distal end of the outer perianth parts, as previously illustrated (3, Fig. 2, B). Leaves are often flecked or striped with white or gray. The leaf symptoms recorded in the current season from inoculations with lily complexes (Table 2) were of this type. A striking effect in tulip, produced by the most virulent strain of cucumber virus thus far isolated from lily, is shown in figure 1. Symptoms were conspicuous 30 days after inoculation and bore a close resemblance to those illustrated by Ainsworth (1) in a tulip naturally infected with a strain of cucumber virus 1 in England.

No attempt is made to classify the symptoms developed by viruses that, for convenience, have been assigned to the "tulip group" (2, 3). More information is needed before these symptoms can be satisfactorily interpreted. The name "latent virus of lily" is applied provisionally to a number of isolates that resemble the virus so designated by McWhorter (12). Such isolates from lily fail to infect tobacco but produce symptoms in tulip and *Lilium formosanum*. There is no assurance that viruses so indefinitely characterized form a related group, but it is convenient to treat them as such a group until differences are found that make such an assumption untenable.

The successful production of current-season symptoms in this series (Table 2) is attributed to inoculation at an early stage of growth rather than to the method, although the leaf-rubbing method is especially convenient to apply to tulips in early stages of development. In view of Gigante's report (7) of current-season symptoms following early injection of virus extract by capillary tube, it is to be expected that early inoculation by hypodermic needle would be successful also. Evidently, previous trials at this station (2, 3) as well as by McWhorter (11, 12) were made too late in the season to permit current-season expression.

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LITERATURE CITED

1. AINSWORTH, G. C. A note on certain viruses of the cucumber virus 1 type isolated from monocotyledonous plants. Ann. Appl. Biol. 25: 867–869. 1938.
2. BRIERLEY, P. Prevalence of cucumber and tulip viruses in lily. Phytopath. 30: 250–257. 1940.

3. ————, and S. P. DOOLITTLE. Some effects of strains of cucumber virus 1 in lily and tulip. Phytopath. **30**: 171–174. 1940.
4. ————, and M. B. MCKAY. Experiments with aphids as vectors of tulip breaking. Phytopath. **28**: 123–129. 1938.
5. CAYLEY, D. M. ''Breaking'' in tulips. Ann. Appl. Biol. **15**: 529–539. 1928.
6. ————. ''Breaking'' in tulips. II. Ann. Appl. Biol. **19**: 153–172. 1932.
7. GIGANTE, R. La variegatura del tulipano. Boll. R. Staz. Patol. Veg. Roma **18** (n.s.): 429–454. 1938.
8. MCKENNEY HUGHES, A. W. Aphis as a possible vector of ''breaking'' in tulip species. Ann. Appl. Biol. **17**: 36–42. 1930.
9. ————. Aphids as vectors of ''breaking'' in tulips. Ann. Appl. Biol. **18**: 16–29. 1931.
10. ————. Aphids as vectors of ''breaking'' in tulips. II. Ann. Appl. Biol. **21**: 112–119. 1934.
11. MCWHORTER, F. P. The antithetic virus theory of tulip breaking. Ann. Appl. Biol. **25**: 254–270. 1938.
12. ————. A latent virus of lily. Science (n.s.) **86**: 179. 1937.

CALCIUM DEFICIENCY AS A FACTOR IN ABNORMAL ROOTING OF PHILODENDRON CUTTINGS

C. M. TUCKER AND P. R. BURKHOLDER

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During the summer and autumn of 1939 considerable trouble was experienced in a greenhouse in St. Louis County, Missouri, in securing normal, healthy, potted philodendrons (probably *Philodendron giganteum* Schott.). In this greenhouse the usual propagation methods were followed. Cuttings were taken from apparently normal plants growing in soil benches and placed in shallow benches of a rather coarse river sand for rooting; following development of roots and the growth of a leaf from the axillary bud, the cuttings were transplanted to pots of soil for marketing.

Symptoms of abnormal development appeared in many of the cuttings after 2 to 4 weeks in the rooting bench; the single leaf on each cutting showed some loss of turgidity and a slightly wilted appearance, accompanied or followed soon by a slight degree of yellowing of the lamina, which was general or confined to localized areas, usually fan-shape and bounded by veins; in more advanced stages necrotic, brown areas developed in the conspicuously yellow portions. The discoloration of this leaf destroys the sale value of the cutting. The axillary bud of affected cuttings grew very slowly, if at all, and sometimes failed to grow, even after 2 months in the sand bench.

The final stage was a dark brown rot of the lower internode, beginning at the cut end below the surface of the sand. As the rot progressed upward and involved the node the cutting was killed.

The roots of the abnormal cuttings presented a characteristic, stunted appearance. They were short, usually 1 to 2 in. long, and produced fewer branch roots than is usual with normal roots; they were brown, but the browning was confined to the superficial tissues. The tips were blunt, sometimes slightly swollen and knoblike, and brown, like the older portions of the roots (Fig. 1). The root hairs were collapsed, brown, and obviously nonfunctional.

Examination of sections of roots failed to reveal invasion by bacteria or fungi, and platings of tissues from roots and rotting internodes yielded only the usual saprophytic, soil-inhabiting organisms. It was apparent that invasion by the fungi recovered occurred following the death of the tissues. The healthy condition of the parent plants from which the cuttings were taken suggested that the abnormal development was not attributable to a virus.

The poor development of stunted, browned roots, the absence of functioning root hairs, and the very gradual development of the symptoms on the leaf suggested failure of absorption by the root system.

In order to determine whether the abnormal appearance of these cuttings may have been related to some mineral nutrient deficiency, several experiments were performed in the greenhouse of the Department of Botany, University of Missouri, in the winter of 1939–1940. In these experiments cuttings were placed in nutrient sand in 3-gal. glazed porcelain pots. Different kinds of nutrient solutions were supplied daily to the cultures. In one series normal fresh cuttings were used. In another experiment cuttings that had rooted unsatisfactorily in a commercial greenhouse were employed. The latter plants bore yellow leaves and the typical peculiar, stunted, brown type of root system.

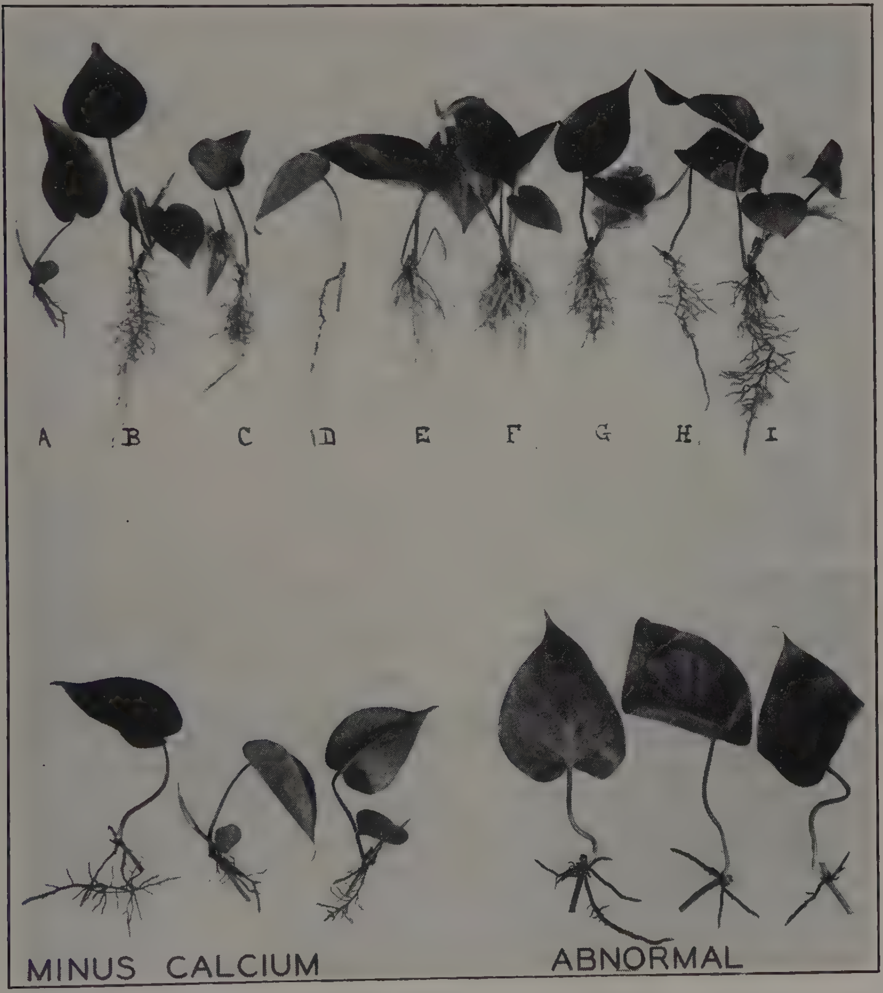

FIG. 1. *Philodendron* cuttings rooted in sand supplied with different kinds of solutions. *Above:* A, watered with nutrient solution minus calcium; B, complete nutrient solution; C, minus potassium; D, minus nitrogen; E, minus phosphorus; F, minus sulphur; G, minus manganese; H, minus iron; I, minus magnesium. *Below:* Left, stunted appearance of roots on cuttings watered with nutrient solution lacking calcium; right, abnormal plants grown under inadequate conditions in propagating bed of commercial greenhouse. (Compare figure 2.)

The composition of the nutrient solution used in the experiment with abnormal, rooted cuttings was as follows: $Ca(NO_3)_2$, 0.002,5 M; $MgSO_4$, 0.005 M; KH_2PO_4, 0.003,5 M. Solutions that were made deficient in one essential element were prepared by substituting another in its place. The salts used in making these replacements were the following: minus N, $CaCl_2$; minus Ca, $NaNO_3$; minus S, $MgCl_2$; minus Mg, Na_2SO_4; minus K, NaH_2PO_4; and minus P, KCl. Trace elements were added to all solutions at the following rates in ppm.: Fe, 0.5; Mn, 0.11; B, 0.05; and Zn, 0.07.

The abnormal cuttings, taken from apparently healthy stock, had been planted in wet, coarse sand in a commercial greenhouse in St. Louis County. Early in November these cuttings with their brown, short roots were transplanted into silica-sand cultures, supplied with the controlled types of solutions. The cultures were kept in moderate shade at a temperature of about 75° F., and solutions were supplied every other day at the rate of about 1 pint per pot. After 6 weeks, all plants were removed from the culture vessels, and examinations were made for comparative development of shoots and roots.

The appearance of typical plants taken from each culture treatment is shown in figure 1. In the upper part of this figure, it may be observed that the root system of plant A, taken from the "minus calcium" treatment, is distinctly inferior to the controls and all others in the series. Under the conditions of this experiment, the various essential elements, with the exception of calcium, appear to have been present in the cuttings in amounts sufficient to permit fairly good growth of roots. Calcium deficiency symptoms were noted in all plants grown in "minus calcium" solution. In the lower part of figure 1, "abnormal" plants, taken from the propagating bench of the commercial greenhouse, are shown for comparison with the "minus calcium" plants taken from the sand culture supplied with solution deficient in calcium; the "abnormal" plants are typical of the cuttings used in this experiment. The photographs indicate something of the external similarity in size, color, and texture of the roots of abnormal and calcium-deficient cuttings; these characteristics were very striking in the living material.

Further confirmation of the calcium deficiency in the abnormal cuttings was given by prepared longisections through the roots of "minus calcium" and "abnormal" plants. The photograph of typical sections (Fig. 2) from deficient and abnormal roots shows the kind of disorganization of meristematic tissue characteristic of Ca deficiency in both. It seems highly probable, therefore, that the philodendron cuttings that showed unsatisfactory growth in commercial greenhouse practice were suffering from inadequate supply of calcium. It is possible, also, that other conditions were not favorable for growth, but the prompt recovery of abnormal cuttings following transplantation into nutrient sand culture would seem to indicate that direct physiological factors were causing the abnormality rather than pathological conditions brought about by a virus or microorganism.

Another experiment was started with fresh cuttings on February 17, 1940, for the purpose of testing various hypotheses concerning the influence of different factors on cuttings, such, for example, as the possible presence of toxic matter in the rooting medium, the effect of moisture supply, texture of rooting medium, and kind of solution employed. Coarse, brown, river sand from the commercial greenhouse, and medium fine silica sand were used as media in 3-gal. glazed pots. Both types of sand were watered with the following kinds of solutions: distilled water, tap water from St. Louis, tap water from Columbia, full nutrient solution, and minus-calcium solution. One whole series was set up in pots provided with drainage through a hole at the bottom, and another in pots without bottom drainage. About a pint of the different liquids was added daily to the drained pots, and small amounts to the closed vessels, as appearance of the sand seemed to indicate requirements for water. It was found that the lower part of the sand was filled with water, even though the surface seemed only moderately moist. Observations were made on appearance of the shoots and roots after 1 and 2 months.

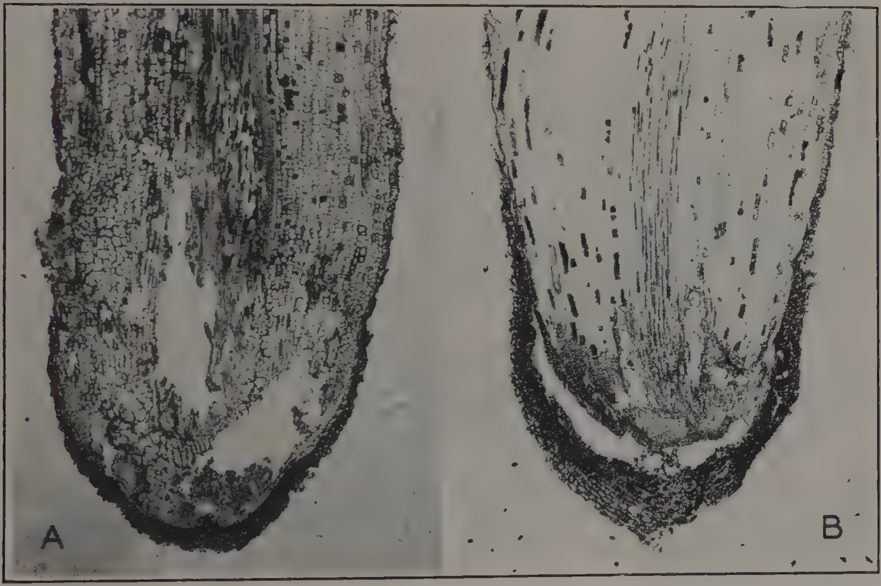

FIG. 2. Longisections through root tips of *Philodendron* showing disintegration of cells in meristematic region. A, section through a root grown in nutrient sand culture with a solution lacking calcium; B, a root grown under inadequate conditions in propagating bed of commercial greenhouse shows similar abnormal structure. (Compare figure 1.)

Several conclusions can be drawn from the results of this experiment, though the water supply was not controlled so carefully as might have been desired. Rooted cuttings of normal appearance were obtained in both kinds of sand, and with both kinds of tap water, and with nutrient solution. Even with distilled water added to the coarse sand, which probably contained some available minerals, in closed vessels, root development was good. Cultures supplied with distilled water and open bottom drainage yielded poor root systems, possibly because of the leaching action. The minus-calcium treatment gave poor, typically brown roots. In general, the cultures with fairly high moisture conditions, whether supplied with tap water or full nutrient solution, produced the best results. The experiment indicates, furthermore, that there was nothing in the water or sand used by the commercial greenhouse that was injurious to the cuttings. It does seem probable that with a fairly coarse sand some special provision needs to be made to insure adequate supply of water and calcium to philodendron cuttings. Rapid rooting and healthy growth of cuttings were obtained within 4 weeks by using moderately fine sand watered with dilute nutrient solution.

SUMMARY

Abnormal development of philodendron cuttings under greenhouse culture resulted from failure to produce effective root systems.

Abnormal, rooted cuttings placed in controlled sand cultures receiving a complete nutrient solution or one lacking either nitrogen, calcium, sulphur, magnesium, potassium, or phosphorus, recovered and developed good root systems; but abnormal, rooted cuttings, transferred to sand cultures and supplied with minus-calcium solution, showed no improvement over their original condition.

Fresh, healthy cuttings, placed in minus-calcium cultures, developed typical brown, stunted root systems.

Under low nutrient conditions the maintenance of fairly high moisture in closed vessels promoted better development of roots than occurred with open bottom drainage.

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STUNT DISEASE OF STRAWBERRY[1]

S. M. Zeller and L. E. Weaver

(Accepted for publication December 26, 1940)

Stunt, a disease of strawberry, has been recognized in the Pacific Northwest for a number of years. It was doubtless confused with yellows[2] (xanthosis) in western Oregon and Washington as early as 1925. The disease does its most severe damage under the cooler, more humid conditions that prevail about sea level along the coast or somewhat inland. In Oregon it occurs scatteringly throughout the Willamette Valley, mostly in the lower or northern part, and down the Columbia Valley. It also has been observed in western Washington.

Stunt is most characteristically expressed in the Marshall variety. Atypical symptoms are found in seedling strawberries, especially in thick dark-leaved sorts. Plantings usually show little of the disease during the first year. Due to decreased vitality and general stunting, the affected plants yield lightly. The fruit is inferior in size and quality. As a rule high percentages of the disease are not found.

The one typical symptom of all affected plants is stunting (Fig. 1). In fields where plants are grown in matted rows stunting shows up most characteristically. Here the dwarfed clones are in sharp contrast to neighboring healthy ones, which are 2 to 3 times as tall, and more robust. Clonal effects indicate the infection of young plants through runners from the diseased mother plant.

The surprising feature of this virosis is the full color of the plants. There seems to be little if any reduction in chlorophyll. Most difference in color between healthy and stunted plants comes from the exposure to general view of the under surface of leaves of infected plants, lending to the whole clone a grayness similar to that resulting from strawberry mildew. This grayness is somewhat exaggerated by the flat dullness of the upper surface of the leaves. The plants grow with considerable erectness, not to the extent found in witches broom[3] but in contrast to the flatness seen in plants affected by yellows (xanthosis) or severe crinkle.

The diseased leaves, typically found in the Marshall variety, are erect and folded along the midvein of each leaflet. The midveins may be somewhat tortuous. The erectness of the leaflets persists until full length of the petiole is attained, then the leaflets open out to expose a flat-dull upper surface. The leaflets may now be somewhat cupped or, more often, with the margins turned downward. The mature leaves make a somewhat papery rattle when brushed by the hand. They are about normal as to color. Any observed lack of chlorophyll has been in the extreme margins. Here, however, the greenness may be only slightly lighter than in normal leaves.

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[1] Published as Technical Paper No. 366 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

[2] Plakidas, A. G. Strawberry yellows, a degeneration disease of strawberry. Phytopath. **16**: 423–426. 1926.

[3] Zeller, S. M. Preliminary studies on witches broom of strawberry. Phytopath. **17**: 329–335. 1927.

In some thick, dark-leaved varieties extreme "cupping" results from stunt infection. In such cases each leaflet may form a perfect cup, almost hemispherical in outline.

Petioles of diseased plants are normal in appearance, except as to length, which is from ½ to ⅔ that of normal petioles. Petioles are erect, however, not prostrate, as they are in the yellows and yellow-edge diseases.

Fruit from diseased plants is small and usually seedy and hard. The roots appear quite normal.

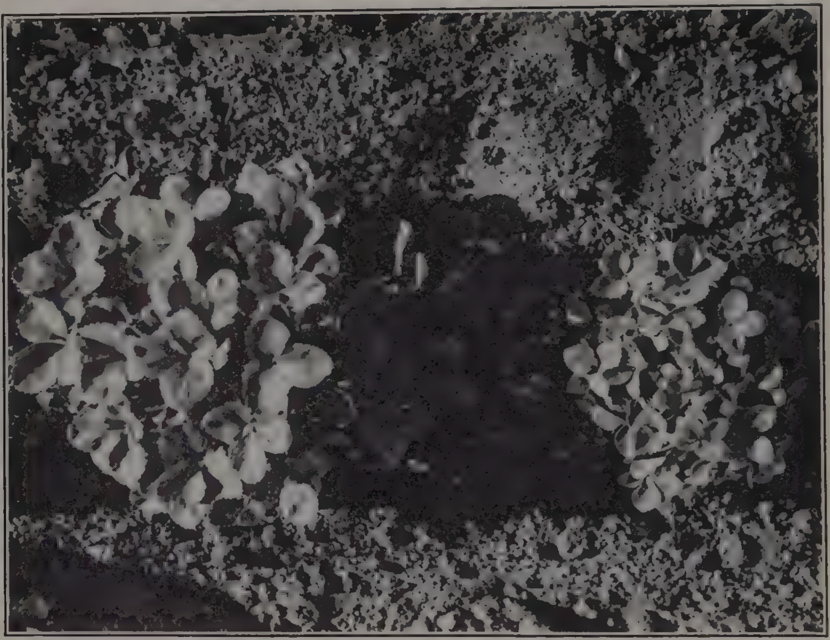

FIG. 1. The Dorsett variety of strawberry; plant to left, healthy; plant to right, affected with the stunt disease.

The stunt disease is readily transmitted from diseased Marshall to healthy Marshall strawberry plants by means of the strawberry-leaf aphid, *Capitophorus fragaefolii*. This experiment was repeated several times in the greenhouse, as well as transmission from certain U.S.D.A. seedlings (resulting from crosses of named varieties of strawberry), to the Marshall variety.

In one case there was evidence of double infection of stunt and crinkle in the William Belt variety. Crinkle was completely masked, but symptoms were not those usually exhibited by plants infected with stunt. The double infection created symptoms somewhat like those produced by tarsonemid mite. When a runner from the William Belt plant was grafted to a runner from a healthy Marshall plant the terminal plants on the grafted Marshall runner developed symptoms of stunt. Young plants on another runner from the parent Marshall developed crinkle and the latter finally showed crinkle symptoms. This indicates that the crinkle virus, as has been shown many times, may travel backward through a stolon, whereas, under the conditions of this experiment, the virus causing stunt traveled forward only toward the distal end of the runner. The symptoms of stunt evidently predominate over severe crinkle in double infections, but then the stunt symptoms are not typical.

Stunt shows rather early in the spring in the Marshall variety of strawberry, especially in plants infected before the current season. As a rule, however, symptoms in most varieties do not appear until late summer or fall, and then they are not readily identified.

The disease is perhaps more closely related to witches' broom than to any other virus disease of strawberry. In some varieties of the host it has some symptoms in common with the yellow-edge disease of England[4] but it is not like yellows[5] in these same host varieties. Some seedlings resulting from crosses between named varieties when infected with the stunt disease take on a dwarfed or stunted condition much the same as plants infested with root weevils. The one distinction between weevil-infested plants and those affected with stunt may be a cupping of the leaflets.

Doubtless the low percentage of this disease found in commercial plantings of strawberries results from eradication through roguing. Growers may readily observe these stunted plants and naturally hoe them out. Control is obtained by careful roguing.

We are proposing ''stunt'' as the common name for this disease, and. even though Fragaria virus 4 has been previously assigned to strawberry dwarf, a disease caused by a nematode, in order to obviate confusion, stunt will be designated as Fragaria virus 5. For those who desire a name of the Holmes type, *Nanus cupuliformans* may be used.

SUMMARY

A new virosis of the strawberry, which stunts plants and causes a cupping of leaves without appreciable reduction of chlorophyll, is transmitted by grafting of stolons and the intervention of *Capitophorus fragaefolii.* The common name, stunt, is used to designate the disease, and the virus is referred to as Fragaria virus 5.

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[4] Harris, R. V. The strawberry yellow edge disease. Jour. Pom. and Hort. Sci. 11: 56. 1933.

[5] *Loc. cit.*

PATHOGENICITY OF AECIOSPORES OBTAINED BY SELFING AND CROSSING KNOWN PHYSIOLOGIC RACES OF MELAMPSORA LINI[1]

H. H. FLOR[2]

(Accepted for publication January 7, 1941)

The reactions of some of the rust-differentiating varieties of flax, *Linum usitatissimum* L., when inoculated with aeciospores obtained by selfing and by crossing physiologic races, were determined during a study on the inheritance of pathogenicity in the flax-rust fungus, *Melampsora lini* (Pers.) Lév., an autoecious, long-cycle rust. Selfed cultures were obtained by the transfer of pycnial nectar from one haploid infection to another, both having originated from first-generation telia descended, through one or more dicaryotic, clonal urediospore generations, from a single urediospore. Hybrid cultures were obtained by the transfer of nectar from a haploid infection of one race to a haploid infection of a second race, each haploid infection having been derived from first-generation telia descended from a single urediospore isolate of each of the two races.

In most cases the physiologic races of *Melampsora lini* were selfed or hybridized on the variety Bison, which has been susceptible to all physiologic races collected in the field. However, in the cases of races 22 and 24, the selective differential varieties were subjected to inoculation with sporidia. Normal pycnia were produced on Ottawa 770 B and on Bombay following inoculation with sporidia of races 22 and 24, respectively. Race 22 is the only one to which Ottawa 770 B is susceptible, and race 24 has been the only race capable of attacking Bombay. The selfed and hybrid F_1 aecia were usually of limited extent, as they were borne on old leaves. One aecium, however, resulting from the transfer of pycnial nectar of race 6 borne on Bison to a pycnium of race 22 borne on Ottawa 770 B, developed sufficient aeciospores to inoculate 6 differential varieties. In addition, selected differential varieties were inoculated with aeciospores of hybrids between races 22 and 24, as well as with selfed aeciospores of each of these two races. The results of these tests, together with the reaction of the 11 varieties of flax that serve to differentiate the physiologic races of *Melampsora lini* to the parent uredial cultures, are given in table 1.

In tests of the urediospore cultures obtained by selfing parent races 6, 22, and 24 it was found that races 22 and 24 were homozygous for pathogenicity to the 11 differential varieties of flax, and that race 6 was homozygous, except for pathogenicity to Akmolinsk.

In all tests thus far made the pathogenicity of the F_1 selfed and hybrid aeciospores has been identical with that of the urediospore culture derived

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[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

TABLE 1.—*The reaction of differential varieties of flax to inoculation with parent races and with* F_1 *aeciospores produced by inbreeding and crossing known races of Melampsori lini*

Race number or F_1 hybrid	Inoculum	Reaction of differential variety										
		Buda C.I. 270–1	Williston Golden C.I. 25–1	Williston Brown C.I. 803–1	Akmolinsk C.I. 515–1	J.W.S. C.I. 708–1	Pale blue crimped C.I. 647	Kenya C.I. 709–1	Abyssinian C.I. 701	Argentine C.I. 462	Ottawa 770 B C.I. 355	Bombay C.I. 42
6	Parent urediospores	R	S	S	R	I	S–	R	I	I	I	I
22	Parent urediospores	S	S	S	S	I	S	S	S	S	S	I
24	Parent urediospores	S–[b]	S	S	R	I	S–	R	I	I	I	I
22	F_1 aeciospores from Bison	S	S	......	......	I	......	......	......	......	S	......
22	F_1 aeciospores from Ottawa 770 B	S	S	......	......	I	......	......	......	......	S	......
24	F_1 aeciospores from Bison	S–		S	......	......	......	......	......	......	......	......
22 × 6[a]	F_1 aeciospores from Ottawa 770 B	SR	S	S	S	......	......	......	......	I	I	......
22 × 24	F_1 aeciospores from Bison	S	......	S	R	......	......	......	......	......	......	......
24 × 22	F_1 aeciospores from Bison	S	......	......	R	......	......	......	......	......	......	......
24 × 22	F_1 aeciospores from Bombay	S	......	S	R	......	......	......	......	......	......	......

[a] The race placed first (maternal) is the one on whose haploid pustules aecia are produced as a result of transfer to them of pycniospore-containing nectar of the other race (paternal).

[b] Minus sign indicates somewhat less susceptibility than the letter designating the host reaction. The letters signify the following: I, immune; R, resistant; SR, semiresistant; and S, susceptible.

from them. The case of the F_1 hybrid 22 × 6, developed on Ottawa 770 B, is of special interest. The aeciospores resulting from this cross, although borne on Ottawa 770 B, were incapable of reinfecting either this variety or Argentine to both of which the paternal parent (race 6) was nonpathogenic. This inability of the aeciospores to reinfect the host upon which they were produced may be considered homologous with heterocism, although occurring in an autoecious rust. This is comprehensible, however, through a knowledge of the physiologic specialization of the races involved, together with the fact that in the F_1 hybrids, between these races, the factor for avirulence is dominant to the factor for virulence.

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PHYTOPATHOLOGICAL NOTES

Reactions of Napier Grass, Merker Grass, and their Crosses to Helminthosporium Eye Spot.[1]—In another paper[2] eye spot of Napier grass in Hawaii, caused by *Helminthosporium sacchari* (van Breda de Haan) Butler, is described and mention is made of the resistance of Merker grass and certain selections from the crosses Napier × Merker and Merker × Napier to the disease. Four of these selections have been recommended as substitutes for Napier.[3] The purpose of the present paper is to record in more detail the reaction of Napier, Merker, and a number of selections from open-pollinated Napier, from selfed Merker, and from the crosses Napier × Merker and Merker × Napier to the pathogen. The data are presented in table 1 and represent a composite picture obtained from observation of natural infection supplemented by artificial inoculation of healthy plants in the greenhouse. Spores of 10 isolates of the fungus were inoculated simultaneously, and the tests were replicated 3 times; greenhouse results substantiated and confirmed field observations, but symptoms were seldom so severe as in the field.

TABLE 1.—*Reactions of Napier grass, Merker grass, and selections from open-pollinated Napier, selfed Merker, Napier × Merker, and Merker × Napier crosses to eye-spot disease caused by Helminthosporium sacchari*

Plant material	Number of accessions observed	Accessions resistant		Accessions susceptible	
		Few spots on foliage		Many spots on foliage	
		No spots on stem	Few spots on stem	Few spots on stem	Many spots on stem
Napier	4	......	......	......	4
Open-poll. Napier	11	2	4	......	5
Napier × Merker	11	4	4	2	1
Merker × Napier	6	1	......	......	5
Merker × Merker	7	7	......	......	......
Merker	2	1	1	......	......

Merker grass is not immune; lesions appear on the leaves and leaf-sheaths as diffuse, smudge-like discolorations, from which *Helminthosporium sacchari* can be isolated. The stems are seldom affected. That this resistance is inherited can be seen by inspection of table 1. The nature of the resistance is not known, but it is significant that the rind of the Merker stem is much harder than the corresponding Napier tissue.—G. K. Parris and J. C. Ripperton, Hawaii Agricultural Experiment Station, Honolulu, T. H.

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[1] Published with the approval of the Director as Technical Paper No. 82 of the Hawaii Agricultural Experiment Station.

[2] Parris, G. K. Eye spot of Napier grass in Hawaii caused by *Helminthosporium sacchari.* (In press).

[3] Ripperton, J. C. Disease resistant strains of Napier grass. Univ. Hawaii Agr. Ext. Circ. 97. 1940.

Identity and Distribution of Three Rusts of Corn.[1]—In August, 1940, E. C. Stakman collected, as no. 143, a leaf rust of corn (*Zea mays* L.) at Tengo Maria, Peru. A part of this specimen was sent to the Arthur Herbarium through the kindness of John A. Stevenson. The abundance and uniform distribution of the uredia and the presence of minute blackish spots beneath the epidermis appeared atypical and led to the suspicion that the rust was not *Puccinia sorghi* Schw., although so labelled. Upon sectioning, the blackish spots were found to be subepidermal, indehiscent telia (Fig. 1, A) with irregular and usually angular, chestnut-brown teliospores measuring 19–27 × 29–41 μ. The apical wall of the spores was only slightly thickened, in contrast to the much thickened apex of the teliospores of *P. sorghi* (Fig. 1, B). Mesospores were present in abundance. The urediospores were yellowish or golden, with 4 or usually 5 rather indistinct equatorial pores, and measured (21-)23–29 × 29–36 μ, thus longer than those of *P. sorghi* (23–29 × 26–32 μ). Such morphological features excluded *P. sorghi* from further consideration, but indicated clearly that the rust was *Puccinia polysora* Underw., a species not previously reported as parasitizing corn.

A careful review of the material deposited in the Arthur Herbarium as *Puccinia sorghi* demonstrated that the Peruvian record was not unique. The oldest specimen of *P. polysora* on corn at my disposal is Farlow's collection from Newton, Mass. This was issued by Ellis in his North American Fungi no. 268 as *P. sorghi*. No collection date appears on the label, but Century III was issued in 1879. In addition to the specimens cited from Peru and Massachusetts, there are also specimens on corn in the Arthur Herbarium from British Honduras, Costa Rica, Cuba, Georgia (Barth., N. Am. Ured. *2563* as *P. sorghi*), Guatemala, Mexico, Mississippi (Barth., N. Am. Ured. *2368* as *P. sorghi*), Panama, Puerto Rico, and Texas. The species was collected also on teosinte (*Euchlaena mexicana* Schrad.) in Guatemala by Kellerman, although previously also considered to be *P. sorghi*.

These discoveries do not necessitate changes in the published characterizations or in the recorded general distribution of *P. sorghi*.

Although *Puccinia polysora* presumably is heteroecious, there are no recorded field observations to indicate the possible aecial stage, nor does the distribution of the species of *Aecidium* furnish any clew. The rust probably is not obligately heteroecious in all of its range, but might logically be expected to complete its cycle in the northern areas.

A third rust (*Angiopsora zeae* Mains) of corn is somewhat similar in appearance in the uredial stage to *Puccinia polysora*. It has been confused at least once (Kern, Whetzel, and Toro *2410* from Puerto Rico) with *P. polysora*, but the sori tend to occur in small groups. The urediospores also differ in that they are smaller (16–20 × 22–30 μ), more finely echinulate, and nearly colorless. In the telial stage, it is readily separable because the unicellular teliospores (Fig. 1, C) are catenulate in sessile chains of usually 2 spores.

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[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

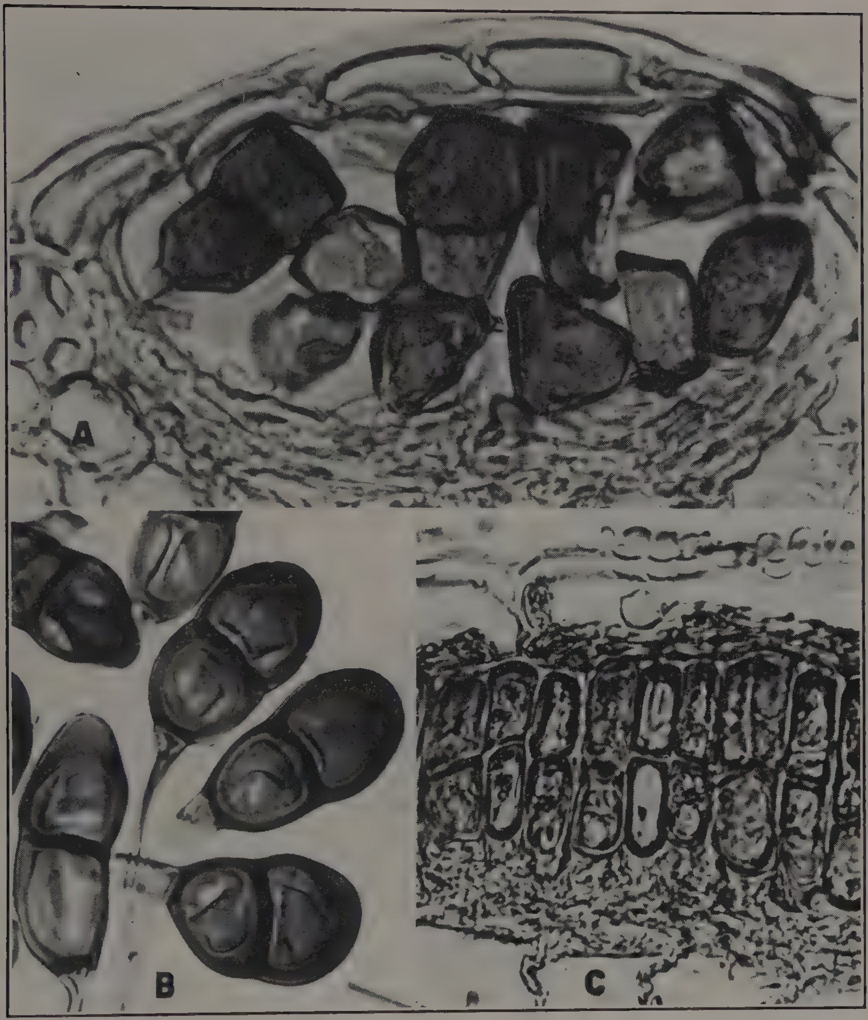

FIG. 1. A. *Puccinia polysora* Underw.; photograph of a free-hand, unstained section showing the indehiscent, subepidermal sori and the angular, thin-walled spores, which distinguish this species from *Puccinia sorghi.* (From Stakman no. 143.) ×800. B. Teliospores of *Puccinia sorghi* Schw. (From type.) ×800. C. *Angiopsora zeae* Mains; photograph of a free-hand, unstained section of a telium showing the subepidermal position and the catenulate, 2 spore chains characteristic of this rust. (From type.) ×650.

Perhaps *Angiopsora zeae* could occur as far north as the United States, but it is known only from Guatemala, Puerto Rico, Santo Domingo, and Trinidad.—GEORGE B. CUMMINS, The Arthur Herbarium, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

Control of Damping-off of Broadleaf Seedlings.—In the study of damping-off of broadleaf seedlings in Federal nurseries of the Great Plains region, the writer has investigated the effect of rotation and previous crops on the control of the disease. *Rhizoctonia solani* Kühn and *Pythium ultimum*

Trow. were the most commonly identified fungi causing damping-off of the broadleaf species.

In both field and greenhouse tests damping-off of broadleaf seedlings was usually more severe following legumes than when cereals were grown as preceding crops. Corn, wheat, and oat soil have given the least damping-off among cereals. This agrees with the findings of Coons and Kotila[1] that cereal rotations, particularly corn, gave a lower incidence of damping-off of sugar beets than legumes, such as alfalfa and sweet clover.

Chemical analyses for soluble nitrogen in the form of nitrate nitrogen by the phenoldisulphonic acid test, showed that the trend of damping-off increased directly with the nitrate content of the soil. For best results agronomic crops should be turned under at least one month before the broadleaf seed is sown. No analyses for total nitrogen, nitrite, or ammonium nitrogen were made, since variations in these are not readily detectable.

Means of decreasing or tying up the soil nitrates by the addition of various materials to soil were also tested. The most outstanding reduction in damping-off was obtained by the application of glucose to the seed bed at the time of sowing. Preliminary field tests with American elm (*Ulmus americana* L.) in a sandy loam soil gave a final stand of 293 per cent on the glucose-treated plots as compared to the untreated checks. Soil analyses made two weeks after treatment showed that nitrate nitrogen had decreased markedly. The seedlings grown on the glucose-treated plots, however, were somewhat stunted at the end of the first growing season. Similar treatments of silt-loam soil were less successful.

Hartley[2] early pointed out that damping-off of pine increased directly with increase in soil nitrogen. Young, Davis, and Latham[3] later found that ammonium compounds, as well as nitrates, increased the damping-off hazard for conifers. The decrease in nitrate nitrogen following treatment of the soil with sugar also helps to explain the earlier success reported by Hartley and Pierce[4] from the application of sucrose to coniferous seed beds in Midwest nurseries.

The application of sugar to the soil probably accelerates the growth of soil organisms, including fungi, which rapidly consume or bind the nitrates until the carbon supply is depleted. The virulence of the damping-off parasites is apparently affected and damping-off losses are reduced. Details of these tests on the control of damping-off broadleaf seedlings will be published later.—ERNEST WRIGHT, Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Lincoln, Nebraska, in cooperation with the University of Nebraska.

[1] Coons, G. H., and J. E. Kotila. Influence of preceding crops on damping off of sugar beets. (Abstract) Phytopath. 25: 13. 1935.

[2] Hartley, Carl. Damping-off in forest nurseries. U. S. Dept. Agr. Bull. 934. 1921.

[3] Young, George Y., W. C. Davis, and D. H. Latham. Control of damping off of conifers. (Abstract) Phytopath. 27: 144. 1937.

[4] Hartley, Carl, and Roy G. Pierce. The control of damping-off of coniferous seedlings. U. S. Dept. Agr. Bull. 453. 1917.

REPORT OF THE 1941 ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The twenty-fifth annual meeting of the Pacific Division of The American Phytopathological Society was held at the California Institute of Technology, Pasadena, California, from June 18–21, 1941, in conjunction with the regular meeting of the Pacific Division of the American Association for the Advancement of Science. Fifty-two members registered and 29 volunteered papers were presented at 4 half-day sessions. Wednesday morning was devoted to a symposium on "Micronutrient deficiency diseases of plants" with the American Society of Plant Physiologists, American Society for Horticultural Science, and Western Society of Soil Science. On Thursday night the society held a dinner with entertainment provided by the membership. Saturday was devoted to a field trip under the direction of K. F. Baker, and diseases of citrus, truck crops, and ornamentals were observed.

Officers of the society for the ensuing year are as follows: President, R. B. Streets, University of Arizona, Tucson; Vice-President, L. D. Leach, University of California, Davis; Secretary-Treasurer, C. E. Yarwood, University of California, Berkeley; and Councilor, N. J. Giddings, U. S. Department of Agriculture, Riverside.

The next annual meeting is scheduled for Salt Lake City, Utah, in June, 1942.

Titles and abstracts of papers presented at the meeting follow.

C. E. YARWOOD,
Secretary-Treasurer

Failure of Rose Bud-unions Caused by Chalaropsis thielavioides.—KENNETH F. BAKER and H. EARL THOMAS.—During the dry summer months of 1940 *Chalaropsis thielavioides* largely prevented successful union of rose bud shields with Manetti and Odorata rootstocks in commercial fields in the Santa Clara Valley of California. In repeated trials, the buds quickly were killed and blackened with only limited discoloration of the rootstock. Fungous contaminants quickly obscured the pathogen which was found to be identical with that causing graft failure of rose in New York. Peyronel's *Chalaropsis* from lupine, Hamond's walnut strain, and Bliss' *Ceratostomella* from date roots were non-pathogenic to Manetti stems. In laboratory tests the rose strain was pathogenic to stems of *Rosa odorata, R. chinensis* var. *Manetti, R. multiflora, R. longicuspis, R. nutkana, R. californica, R. gymnocarpa,* sweet cherry, peach, almond, black and Persian walnut, pear, and *Prunus mariana,* and grew slightly on orange and quince stems and *Ulmus pumila* roots, but stems of *R. laxa,* apple, and numerous collections of the Ragged Robin rose were immune. *Rosa odorata* is the most susceptible rootstock, followed by Manetti and *R. multiflora.* The disease is not known in southern California, where roses are largely of the garden type grown on immune Ragged Robin rootstocks. Because practically all California-grown rootstocks are budded before shipment, the problem is distinct from that in the east.

Artificial Inoculation of Plants with Armillaria mellea.—DONALD E. BLISS.—Infection with *Armillaria mellea* was obtained only by means of rhizomorphs that were attached to a suitable substratum. Detached rhizomorphs were non-infective. When pure cultures on nutrient agar, bran, and wood were placed in soil about normal citrus roots, infection resulted only from the woody inoculum. Disease transmission was obtained both from naturally and artificially infected wood. Best results were obtained from pure cultures grown on sterile segments (20 cm. in length and 3–5 cm. in diameter) of citrus roots, the potency of the inoculum depending upon adequate food reserves in the wood. Rhizomorphs of *A. mellea* developed in soils differing widely in type and reaction—in such soils as peat moss (pH 4) and sand (pH 8), for example. The rhizomorphs penetrated rotted, non-sterile manure, but apparently did not attack it as a source of food. Within 12 months, rhizomorphs grew to a depth of 67 cm. in sterile glucose-potato agar; but, when air was excluded from the surface, growth stopped immediately and the fungus died. *Trichoderma lignorum* suppressed the growth of *A. mellea* on agar but did not destroy it in wood after a pseudosclerotium had formed. *Schinus molle* (California pepper tree) was infected after 71 days in relatively moist soil at 20° C.

Studies on Peach Viroses in Idaho.—EARLE C. BLODGETT.—A peculiar peach disease reported in Idaho in 1938 has been named "wart." Symptoms are characterized by warty out-growths on the fruits, particularly near the stylar end. No tree or foliage symptoms are recognized. When buds from warty trees are placed on healthy trees, the fruit on the stock shows the disease. Additional work is under way, including host range and variations in symptoms on peach known as "smooth wart" and "crease wart." A peach disease characterized by a mottle pattern of the foliage has been known in Idaho since 1939.

The disease is regarded as different from mosaic and is called peach mottle. Budding tests indicate that mottle is easily transmitted from diseased to healthy peach, and buds from mottle peach trees placed on healthy Bing cherry cause a disease similar to cherry mottle leaf. Limited trials also show that when buds from diseased peach are set on healthy Montmorency, the trees exhibit severe dieback of current growth. A peach disease, common in Idaho orchards, causing yellowing of foliage and usually attended by necrosis of irregular areas of leaf tissue and finally defoliation, is regarded as a virosis similar to the X disease.

The Effect of Aluminum on the Deposition of Copper on Cucumber Leaves and on Protection Against Peronoplasmopara cubensis.—JAMES F. L. CHILDS.—Particulate copper fungicides were found to have their negative charge replaced by a positive charge in the presence of aluminum nitrate. The fungicidal action of fungicides sprayed on plant surfaces indicated that increasing amounts of aluminum nitrate added to copper phosphate, and 3 proprietary cuprous oxide (Cuprocide) suspensions increased their protective capacity against *Peronoplasmopara cubensis,* the downy mildew of cucumber. The protective action of graduated concentrations of aluminum nitrate in distilled water showed a correlation of protective capacity with aluminum nitrate concentration against *P. cubensis,* similar to that exhibited by the cuprocides and copper phosphate. Colorimetric analysis indicated that on cucumber leaves the deposition of copper phosphate and Cuprocide H–112 (acid suspensions) was decreased in the presence of aluminum nitrate, but the deposition of Cuprocide H–167 (alkaline suspension) was not affected. These data are believed to indicate that the increased protective capacity conferred by aluminum nitrate on the fungicides tested is more closely related to an intrinsic property of the aluminum nitrate than to the effect of the aluminum nitrate on the charge and consequent deposition of the fungicide particles on the cucumber leaves.

A Severe Ring-spot Virosis on Peach.—L. C. COCHRAN and LEE M. HUTCHINS.—In May, 1940, peach mosaic inspectors called the authors' attention to orchard peach trees of J. H. Hale and Late Elberta showing twig blight and severe dieback; the 1940 growth displayed dark sunken areas of various sizes from spots to large cankers; scattered leaves variously showed rings, yellow spots, and chlorotic patterns. In May, 1941, none of these symptoms were present on the trees affected in 1940. On May 15, 1940, J. H. Hale peach nursery trees received graft-inoculum from the severely affected orchard trees. Within 2 months after inoculation, the test trees displayed sunken areas in the bark and dying of twig terminals in vertical line with the points of inoculation. By October, 1940, the test trees showed severe twig girdling and severe splitting and furrowing of bark on the trunks. In 1941, the test trees were severely retarded in starting growth; initial leaves bore rings, spots, and mottled pattern like those seen in the orchard trees in 1940; the developing foliage was abnormally pale-green; most of the mottled leaves abscissed by May 15 and the later foliage appeared normal. Nursery trees of Ne Plus Ultra almond, myrobolan plum, mahaleb and mazzard cherry, graft-inoculated from the affected orchard peach trees, developed ring spots and chlorotic and necrotic patterns in the leaves. Other transmission tests from widely separated localities have demonstrated the existence of a virus group in stone fruits in California capable of producing ring spots and symptoms in peach comparable to those described here.

Resistance and Susceptibility to Curly Top in Varieties of Cucurbita pepo and C. moschata.—B. F. DANA.—Commercial seed strains from North America and abroad and miscellaneous strains received through the U. S. Division of Exploration and Plant Introduction were exposed to natural infection from curly top on the Umatilla Field Station, Hermiston, Oregon. Test plots were carried on cooperatively by the U. S. Department of Agriculture and the Oregon Experiment Station from 1928 to 1940. Extreme susceptibility to curly top was general in varieties in the Patty Pan and Crookneck-Straightneck groups of *C. pepo.* Resistance was evident in Long White Bush and Boston Greek vegetable marrows and Big Tom pumpkin. Otherwise, intermediate resistance was general in varieties of the vegetable marrow, fall, or individual and winter pumpkin groups of *C. pepo.* Very strong resistance approaching immunity in most varieties was characteristic of all the varieties of *C. moschata.* Only in rare cases and under conditions of drought and high insolation did injury from curly top appear in these varieties. This is suggestive of a possible relationship between strong resistance and vegetative vigor coupled with the late-blooming or long-season habit of varieties in this species.

Symptoms of Nutrient Deficiencies and Excesses in Citrus.—L. J. KLOTZ and J. C. JOHNSTON.—*Vein chlorosis* of leaves results from root injury, as by girdling and drowning. *Nitrogen starvation* shows in general yellowing of leaf. In *iron chlorosis* yellow leaves retain green veins until late stages of albescence and burning. *High pH* decreases minor element availability, retarding growth. *Copper deficiency* is recognized by some of the following: gum-pockets at leaf nodes; resinous excrescences on twigs and on elongated leaves and fruit; gum in angles of fruit segments; multiple buds, staghorn growth, S-shape terminals. *Zinc-deficient* leaves have irregular yellowing between lateral veins; severe cases bear undersize leaves, pale, coarse fruit, multiple buds, dieback. While similar in pattern, *manganese deficient* leaves are full-size, light-green between veins, frequently bearing small, circular, chlorotic spots. The green wedge along midrib of chlorotic leaf and green tip, said characteristic of *magnesium deficiency,* also has been attributed to excess manganese (spray), seasonal nitrogen deficiency, certain calcium-magnesium ratios; veins may split. *Boron deficiency* kills terminal twigs, splits bark (resembling psorosis B), makes translucent areas in young leaves, curled, brittle, brownish-green, mature leaves with split, corked veins on upper surface, flowers-fruit abscission, fruit with longitudinal fissures in rind surface, gum in albedo, core, angles of pulp segments. *Excess boron* causes golden yellowing and dying at leaf tips and margins, circular resinous spots on dorsal surface.

Resistance of Citrus Tissue and Psorosis Virus A to Heat.—H. S. FAWCETT and L. C. COCHRAN.—In 5 separate trials from 1936 to 1940, Valencia orange twigs with suitable buds for propagation were immersed in water and held at different constant temperatures. Part of these were from trees affected with psorosis A, (*Citrivir psorosis* var. *vulgare*) and others from virus-free trees as checks. The buds were then placed in virus-free nursery trees. Seventy-five different tests with inoculations into 450 nursery trees were made, besides 40 tests based on observations of 240 sticks, buds from which were not placed in trees. Young leaf symptoms in the test trees were used as a criterion of virus transmission. A temperature-time curve for resistance of citrus tissue to heat while immersed in water has been approximated. This curve for psorosis-affected tissue passes close to 60° C. for 1 minute, 55° for 5 min., 50° for 1 hr., and 45° for 10 hr. In only 3 trees among 130, in which psorosis buds lived in the 1940 experiment, have leaf symptoms failed to show. It is thus indicated that this method will be of no direct practical value. Virus-free bud sticks appeared more heat resistant than psorosis-affected ones. In 40 tests, including 240 bud sticks in each of the two lots, 97 psorosis-infected and 79 virus-free were killed.

The Effect of Phosphorus Nutrition on Concentration of Tobacco-mosaic Virus.—S. FRIEND.—The influence of phosphorus nutrition on virus multiplication in Turkish tobacco plants was studied by growing them in nutrient solutions containing different levels of phosphorus, but with a relatively high nitrogen supply. The amount of phosphorus, nitrogen, and potassium absorbed by the plants receiving all elements essential for growth in the nutrient solution was controlled. The plants were inoculated in the 4-leaf stage with tobacco-mosaic virus and the juice from plants harvested 12 to 22 days following inoculation was assayed for virus concentration. Under conditions of high nitrogen nutrition there is a definite range of phosphorus concentration where this element becomes a limiting factor in the multiplication of virus. The increase in virus concentration showed no correlation with the amount of growth of the host.

Curly-top Resistance of Sugar-beet Plants as Influenced by Age.—N. J. GIDDINGS.—Replicated plantings of one susceptible and one resistant sugar-beet variety were made at 3 dates approximately a month apart. The plants in one half of each plot of each variety were inoculated about 1 month after the last planting was made. The other half of each plot was held as a noninoculated check. Curly-top readings were made at approximately weekly intervals. Two months after inoculation the resistant variety U.S. 22 showed about $\frac{1}{6}$ as much curly top as the Old Type variety in the January planting, about $\frac{1}{3}$ as much in the February planting, and about $\frac{1}{2}$ as much in the March planting. The variety U.S. 22 showed a pronounced tendency to recover from curly top, while recovery was much less evident in the variety Old Type. The older beets were far more resistant in each variety, but the older plants of U.S. 22 showed a much greater increase in resistance than those of Old Type.

Histological Studies on the Relationship of Dodder to Healthy and Curly-top-infected Sugar Beets and Tobacco.—C. F. LACKEY.—Bennett found dodder occasionally capable of transmitting the curly-top virus from infected beets and tobacco plants to healthy ones. This would involve a close relationship with the host's phloem in which Bennett found the virus to occur. So far as has been observed dodder haustoria on healthy beet petioles go straight to the phloem tissue. Subsequently if conditions are favorable for growth the haustoria make connections with that of the xylem of the host plant. When a beet plant becomes infected with curly top, degeneration of the phloem sets in. It has been observed that in cases of severe necrosis the haustoria of *Cuscuta californica* did not reach the phloem tissue. These haustoria were stopped in growth and often appeared to be walled off. However, in view of the fact that transmission of the virus from diseased to healthy plants was effected in a few cases, it may be that the haustoria occasionally did make connections with the diseased phloem. The haustorial strands of *Cuscuta californica* on healthy tobacco were found connecting first with the outer and inner phloem. With severely diseased plants the outer phloem degenerated, and cavities were formed between the outer phloem tissue and the rest of the vascular ring. This prevented the dodder haustoria from making contact with the main part of the ring.

The Development and Spread of Pierce's Disease of Grapevines.—WM. B. HEWITT.—Pierce's disease of grapevines, reported transmissible by grafting in 1938, appears to be the same as that named the Anaheim or California vine disease. This malady was first reported in Southern California near Anaheim and North Pomona in 1884; by 1895 it had spread to most of the southern California vineyards and destroyed nearly 30,000 acres of vines. During this time the disease was reported in Napa, Livermore, and northern Sacramento valleys, but was not so destructive as in the southern part of the State. The widespread death of vines in the Santa Clara Valley from 1887–1901 was probably not due to the same disease. The San Joaquin Valley apparently remained free of the disease until some time during the 1920's. In 1930 the death of vines in Tulare County was called to the attention of the University of California, and later identified as similar to the California vine disease. It is probable that this disease has been relatively inactive in the State since 1900, until its recent outbreak in the San Joaquin Valley. Pierce's Disease of grapevines has recently been found in most of the vineyard districts in the State.

The Feeding of Nematodes Before and During Their Entry into Roots.—M. B. LINFORD.—Three nematode species that remain slender, active worms throughout life and 2 in which females become immobile, all begin feeding on the root surface and continue during their entry into roots. The feeding process consistently involves: insertion of the stylet tip into a cell; a period of apparent rest when saliva may be injected; and an equal or longer period of sucking out cell contents. Stylet perforations leave no visible holes in walls. Cells are destroyed chiefly by entry of a nematode after the cell has been fed in and the wall has subsequently been weakened by numerous stylet thrusts. Root-knot nematode larvae (*Heterodera marioni*) may feed repeatedly in young epidermal and dermatogen cells, destroying some, and they feed persistently during intercellular migration to their permanent sites. Meadow nematode (*Pratylenchus pratensis*), *Rotylenchus erythinae*, and reniform nematode (*R. reniformis*) feed on piliferous zone epidermis before their destructive intracellular penetration into, or migration through, the cortex. The latter two then feed in the endodermis without cell destruction. *Paratylenchus sp.* feeds chiefly from epidermal cells and root hairs, less often entering the cortex. Washing a root removes most *Paratylenchus* and *Rotylenchus*, leading to underestimation of their abundance.

Some Soil-moisture Relationships of the Root-knot Nematode.—M. B. LINFORD.—The colloidal but granular Hawaiian soils studied were highly retentive of water, with narrow ratios between moisture equivalent and wilting percentage. Egg masses of *Heterodera marioni* were incubated 10 to 30 days in folds of bolting silk in soil with a moisture equivalent of 39 per cent, and wilting coefficient near 28 per cent. Many eggs died at soil moistures of 23.8 and 25.6 per cent. Most remained alive at 28.6 and 29.8 per cent, with a little slow hatching but with embryo development that permitted rapid hatching when later transferred to water. Half way up to the moisture equivalent, hatching was progressively more rapid; then, to 45 per cent, it occurred as rapidly as in shallow water. Tomato and cowpea were grown short periods from sprouting seeds in large volumes of infested soils at pre-adjusted moisture contents, protected from rapid water loss, and not watered after planting. Few or no larvae entered the ample roots growing near the wilting percentage. With increasing wetness, to somewhat above the moisture equivalent, intensity of infestation and severity of root injury increased greatly. Both declined in still wetter soil.

The Effect of pH on the Inactivation of Tobacco-mosaic Virus by X-rays.—ALFRED MARSHAK and WILLIAM N. TAKAHASHI.—A number of different workers have shown that both inorganic and organic colloids are precipitated by X-rays when they are positively charged and not when negatively charged. In previous experiments the senior writer has shown that the sensitivity of chromosomes to X-rays can be reduced by removing the positive charge on their surface by treatment with dilute ammonia. The experiments with ultracentrifugally purified tobacco-mosaic virus reported here show that on the acid side (pH 2.2) of their isoelectric point, where the particles carry a net positive charge, they are more sensitive to inactivation by X-rays than on their alkaline side, *e.g.*, pH's 4, 5, 6, and 7. It is believed that, since the virus particles carry a greater net positive charge on the acid side of their isoelectric point, the observed greater inactivation at pH 2.2 may be attributed to a more efficient capture of electrons formed in the suspending medium by X-rays. These captured electrons apparently have sufficient energy to bring about the inactivation of the virus.

Crown Rot of Rhubarb Caused by Pythium spp.—JOHN T. MIDDLETON.—Rhubarb, grown in the vicinities of San Francisco and Los Angeles, is subject to a fungus decay in which the roots, crowns, buds, and petioles may be affected. When roots are infected, the plants are usually small and somewhat chlorotic, producing few marketable petioles; such plants often die, though occasionally they recover. Plants with infected crowns are most seriously affected, the crowns becoming soft and water-soaked. Infection progresses from the crown to the bud scales, ultimately infecting the petioles and blighting the young shoots. Petioles pulled from plants with infected bud scales often decay in transit, spreading the infection throughout the container. *Pythium anandrum, P. oligandrum* and *P. ultimum* have been isolated from diseased plants. Each species is capable of causing the disease independently, producing identical symptoms. Although each species upon inoculation is able to cause damping off of rhubarb seedlings, *P. ultimum* is most pathogenic.

Root Rot of Barley Caused by Pythium hypogynum n. sp.—JOHN T. MIDDLETON.—A root rot of barley observed in Missouri caused damage in areas with poorly drained soils. The roots of infected plants were discolored and frequently flaccid from corticular decay. Shoots were usually stunted and chlorotic in appearance. Plants, infected but not killed, rarely stooled or produced heads. An undescribed species of Pythium was consistently isolated from infected roots and proved pathogenic upon inoculation. The following is a specific diagnosis: *Pythium hypogynum* n. sp., mycelium non-septate when young, irregularly septate when old, measuring 1.5 to 8.3 μ, average 5.1 μ in diameter. Sporangium terminal, rarely intercalar, spherical to subspherical, smooth- and thin-walled, measuring 6.5 to 34.6 μ, average 22.1 μ, in diameter, sporangium germinating by means of germ tubes or zoospores. Oogonium terminal, spherical to subspherical, smooth- and thin-walled, measuring 10.2 to 35.1 μ, average 22.0 μ in diameter. Antheridium hypogynous, single, delimited within the oogonial stalk at a distance varying from 5 to 30 μ below the oogonium; antheridial cell small, 2.8 to 8.3 μ, average 5.4 μ, wide, 3.0 to 11.1 μ, average 6.6 μ, long, supplied with a tenuous fertilization tube that just penetrates the oogonial wall. Oospore plerotic, smooth-walled, containing a single reserve globule and a prolate ellipsoidal or flattened refringent body. Oospore germination not observed.

Phytophthora Bleeding Canker of Quercus agrifolia.—P. A. MILLER.—Bleeding canker of the California live oak (*Q. agrifolia*) was first observed in California in the Los Angeles area in the spring of 1938. It has since been found in the adjacent areas of Pasadena, San Marino and has been reported from Santa Barbara. In 1939 and 1940, Howard and Caroselli described a bleeding canker of maple trees in Rhode Island. Beech, elm, and oak trees in the eastern coastal section of the United States are also now known to be affected. Active cankers appear as oozing bark fissures at the root crown and upward at intervals on the trunk. These ''bleeding'' bark cracks indicate the location of fluid-filled, interconnected developing cavities in the underlying inner bark, cambium and sapwood. The consistence and color of the fluid varies from a light brownish water to a thick reddish brown ooze, depending upon the extent of necrosis, decomposition, oxidation, and dehydration of the infected tissues. It dries to black, tarry, or crystalline masses in old cavities or on the bark surface at the exuding cracks. Artificial inoculations of *Q. agrifolia* and *Q. virginiana* with a species of *Phytophthora* isolated from infected sapwood produced characteristic lesions. Bark tracing as a means of control is ineffectual.

Vitamin B_1 and the Development of Cantaloup Powdery Mildew.—DEAN E. PRYOR.—Recently, certain preparations containing vitamin B_1 have been recommended as a control measure for cantaloup powdery mildew, the assumption being that a more vigorous growing melon plant is resistant to mildew. In connection with other work, the effect of thiamin on race 2 of *Erysiphe cichoracearum* has been studied using as a host excised leaves of No. 45 cantaloup in Petri dishes and plants of the same variety growing in soil. With dishes the results indicate that 2 days after inoculation vitamin B_1 caused a statistically significant stimulation of mildew. The significance of this increase was transitory although the trend was maintained for some days after inoculation. In the soil experiments there was no statistically significant effect on growth or on the disease, but here, as with the dishes, the treated plants tended to develop more mildew. From these preliminary results it would seem that thiamin offers little possibility for control of cantaloup powdery mildew.

The Reaction of Cantaloup Strains to Powdery Mildew.—DEAN E. PRYOR and THOMAS W. WHITAKER.—The reaction of cantaloup strains to race 2 of *Erysiphe cichoracearum* described herein was studied primarily under greenhouse conditions. Symptoms exemplified by the infection on plants in an F_2 population were grouped in 5 classes depending upon extent of mycelial development and degree of sporulation, 0 to 4 indicating the range from absence of mycelium to vigorously sporulating mycelium entirely covering the leaf. Some plants showed a faint yellow spotting to a definite severe necrosis, generally with mycelial production restricted or absent. Strains of cantaloup have been isolated that consistently have a 0 reaction, but light to pronounced spotting, the severity of which seems to be genetically controlled. Other lines vary in reaction from 0 to 2, which implies that some factors for resistance are still segregating. Cantaloup No. 45, highly resistant to race 1 of the fungus, does not seem to differ in reaction to race 2 from Hale's Best, an older variety very susceptible to both races of the pathogen. Since some plants in heavily inoculated, segregating populations remain entirely free of all macroscopically visible mildew symptoms, there is hope of isolating even more resistant strains than are now employed in the breeding program.

Tomato Plant Populations in Relation to Curly-top Control.—MICHAEL SHAPOVALOV, H. LORAN BLOOD, and ROY M. CHRISTIANSEN.—Approximately 3,560 tomato plants are set per acre in Utah with the customary 3½-ft. spacing between the hills. Severe outbreaks in curly top often reduce this number by more than 50 per cent. In the experiments conducted over the past 5 years the writers modified the traditional planting density by setting 2 plants in each hill and by reducing the space between hills. Invariably the percentage of curly-top-affected plants was lower and the yields higher with quadruple populations than with either the checks or doubled populations. The reduction in the percentage of disease in the densest plantings varied from 4.4 to 30.0 per cent, and the increase in yield from 2.9 to 9.6 tons per acre. With a given number of plants per acre, a somewhat better control of curly top was obtained with 2 plants per hill than with single-plant hills more closely spaced; however, the yields tended to be higher with single-plant hills. This indicates an extra economic benefit from close spacing in addition to improving stands through curly-top control.

Mortality of the Red Scale on Lemons Through Infection with a Spore-forming Bacterium.—V. P. SOKOLOFF and L. J. KLOTZ.—Adult females of *Aonodiella aurantii* can be destroyed, under controlled conditions, by a denitrifying bacterium isolated from the soil and tentatively designated as Bacillus "C." Immersion, spraying, and dusting with spores were the methods used to bring about mass infection of the insects by the Bacillus. Approximately 7000 insects served as our material. Mortality among the infected lots was 100 per cent under optimum laboratory conditions. Production of gas, distortion of the pygidia, and a characteristic browning of the insects often appeared to be symptoms of the infection. They were followed by disintegration and drying. Bacillus "C" could be detected in the smear preparations of the diseased and dead insects. Extracts of healthy insects contained measurable amounts of nitrate. Extracts of the infected insects showed very little, if any, nitrate. Slight increases in the nitrite content of the latter were insufficient to account for the loss of nitrate. Bacillus "C" is a large motile Gram-positive rod, forming spores in the equatorial position. An aerobe, it can grow anaerobically in the presence of either nitrate or nitrite on a number of organic substances, including chitin.

A Virus Inactivator from Yeast.—WILLIAM N. TAKAHASHI.—A substance capable of inactivating tobacco mosaic virus has been extracted from commercial brewer's and baker's yeast by autolysis and by autoclaving. Its action on virus suspensions is instantaneous at room temperature, and its activity appears to bear a direct relation to its concentration. The multiplication of virus was retarded in inoculated detached leaves having their petioles in a solution of the inactivator. However, when plants were grown in sand culture, the addition of the inactivator to the nutrient solution did not prevent infection. The inactivator can be partially purified by filtering an autoclaved decoction of yeast through celite, then precipitating the active substance with acetone or alcohol. The white precipitate is readily soluble in water and is further purified by clearing the solution with safranin or lead acetate. The active substance is heat-stable and, therefore, is not an enzyme. It gives negative results with protein tests, is not destroyed by trypsin, and is not adsorbed by heat-denatured virus.

Buckskin Disease of Stone Fruits.—H. EARL THOMAS and T. E. RAWLINS.—Two strains of the buckskin virus that produce distinct symptoms in sweet cherry cause similar symptoms in Orange Cling peach but apparently different in intensity. Attempts to induce recovery of affected peach cions by growing them on apricot and myrobalan roots have failed. Cutting the leaf blade and lateral veins of peach leaves does not seem to accelerate or retard the appearance of symptoms. The virus is able to pass from peach to peach through a short piece of leafless myrobalan stem and through apricot and *P. davidiana* stems with leaves attached. Breakdown in the phloem of affected peach and cherry is very similar to that described by Quanjar and others for potato leaf roll. A branch or entire peach tree in the orchard may have symptoms one year, none the following year, with symptoms reappearing in later years. Flowers of infected peach trees are not only retarded, but, in some varieties, are distinctly reduced in size.

Bordeaux Increases Water Loss from Inert Surfaces.—C. G. WEIGLE.—Uniform-size disks of *Nicotiana glutinosa* leaves were paired and killed by steaming. One member was sprayed with water, the other with an equal amount of 1 per cent Bordeaux mixture. After drying the surfaces, subsequent water loss was measured by weighing. Of 194 weighings of such pairs under varying environmental conditions 174 showed that the Bordeaux-treated disks lost water more rapidly than the control disks. The average ratio of loss from the Bordeaux-treated surface to the water-treated surface was 1.42. Similar sprays were applied to paired disks of thin waxed paper that, after drying, were placed on moist filter paper. Subsequent weighings showed the average ratio of loss from the Bordeaux-treated to the water-treated was 1.61 with 8 pairs of disks. A small hole was pierced in heavy waxed paper disks. One disk was sprayed with water, the other with Bordeaux. After drying, these were floated on water that seeped through the hole and spread over the entire surface of the Bordeaux-treated paper. The control remained dry. Water, dropped on dried Bordeaux-sprayed *Eriobotrya* leaves, covered 4 times the measured area than on dried water-sprayed leaves. Thus, it is indicated that capillary properties of dried Bordeaux may be important in interpreting these results.

Stimulatory Action of Egg Yolk in the Culture Medium on Conidia of Powdery Mildews.—C. E. YARWOOD.—Hens' egg yolk, dissolved in 0.1 per cent potassium hydroxide, was the most stimulatory substance tested for increasing the percentage germination and germ-tube length of the conidia of the powdery mildews of barley, bean, rose, live oak, sunflower, California poppy, clover, evonymus, peach, cucumber, grape, lilac, and apple. Increases in percentage germination and germ-tube length, due to egg yolk, varied from slight to infinite, since in many tests the conidia without egg yolk failed to germinate. The optimum medium consisted of about 0.5 per cent egg yolk and 2.5 per cent sucrose in 1.2 per cent agar. Barley mildew showed the best response to egg yolk and yielded germ tubes averaging 150 μ in egg yolk cultures, while control cultures yielded germ tubes averaging 25 μ, but the greatest absolute length of germ tubes observed in agar cultures was with mildew from live oak. Other substances found stimulatory to powdery-mildew conidia were alkali extracts of dried barley leaves, cows' milk, rabbit blood, rabbit liver, iron sulphate, copper sulphate, and brown sugar. Dilute copper sulphate or Bordeaux mixture, applied to bean leaves before inoculation, increased the subsequent development of powdery mildew on these leaves.

Evaluating Sulphur Fungicides for the Control of Downy Mildews.—C. E. YARWOOD.—On dusted glass slides sulphur dust (Flottox) inhibited the germination of the sporangia of the downy mildews of hop and snapdragon in water suspensions, but was apparently not toxic to the sporangia of the downy mildews of onion and lettuce. On agar plates sulphur was toxic to the sporangia of the downy mildews of hop, cucumber, snapdragon, and onion, but not to the sporangia of lettuce mildew. On leaves in moist chambers, sulphur inhibited the germination of the sporangia of snapdragon mildew but did not markedly inhibit germination of sporangia from cucumber, onion, or lettuce. However, sulphur-dusted plants were protected from artificial infection with the downy mildews of hop, cucumber, snapdragon, and lettuce, but not with onion mildew. It is concluded that sulphur dust has protective value against certain downy mildews, and that germination tests are unreliable in evaluating sulphur fungicides. Sulphur dust afforded marked protection from hop downy mildew at 4° C. and from snapdragon rust at 7° C., which are lower temperatures than those at which sulphur usually is considered effective.

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Am 28. Juli, 1939, ist Hofrat o. ö. Prof. Dr. Gustav Köck auf seinem Landsitz in Spital am Semmering, kaum 60 Jahre alt, einem Schlaganfall plötzlich erlegen. Köck war seit dem Jahre 1930 Vorstand der Lehrkanzel für Phytopathologie an der Hochschule für Bodenkultur in Wien, nachdem er vorher durch 25 Jahre an der jetzigen Biologischen Reichsanstalt für Land- und Forstwirtschaft, Zweigstelle Wien, tätig war. Auf seinem Arbeitsgebiet hat der Verstorbene in nie ermüdendem Eifer eine ungemein reichhaltige und vielseitige Tätigkeit entfaltet. Daher ist es nur selbstver-

GUSTAV KÖCK

ständlich, dass der Phytopathologe Köck in den pflanzenschutzlichen Kreisen der Ostmark und des übrigen Reiches, aber auch darüber hinaus im Ausland wohl bekannt ist. Neben zahlreichen Büchern und Broschüren, die er zum Teil Allein zum Teil als Mitverfasser zeichnete und die zur Verbreitung pflanzenschutzlicher Kenntnisse in den Kreisen der Landwirte, Gärtner, Obstliebhaber und Pflanzenfreunde überhaupt ganz wesentlich beitrugen, sind mehr als 150 Arbeiten und Aufsätze, das Gesamtgebiet des Pflanzenschutzes betreffend, das literarische Ergebnis seiner umfangreichen Studien. Darüber hinaus ist seiner Initiative auch die Entstehung einer Reihe von wissenschaftlichen Arbeiten zu verdanken, die während seiner Tätigkeit an der Hochschule für Bodenkultur von Dissertanten angefertigt wurden. Seine beiden Hauptarbeitsgebiete waren die Kartoffelkrankheiten und die Rauchschadensfragen. Als langjähriger Vorsitzender des Kartoffelfachausschusses der ehemaligen landwirtschaftlichen Bundesversuchsanstalten hatte er reichlich Gelegenheit, sich nur mit dem Kartoffelbau vom pflanzenbaulichen Standpunkt aus zu beschäftigen, sondern im besonderen auch auf seinem eigenen Arbeitsgebiet richtungweisend zu wirken. In diesem Zusammenhang sei nur auf eine seiner grundlegenden Arbeiten, (Studien über die Blattrollkrankheit der Kartoffel) verwiesen. Sein anderes Spezialgebiet waren die Rauchschadensfragen. Es gibt wohl kaum einen Rauchschadensstreitfall in der Ostmark, dem nicht Köck als Sachverständiger beigezogen worden wäre. Durch diese seine mehr als 25 jährige Tätigkeit hat er sich eine ungemein reiche Erfahrung in den oft schwierigen Rauchschadensfragen erworben, die ihn in die Lage versetzte, über diese allein begutachtende Tätigkeit hinaus die vielfach komplizierten Rauchschadensprobleme auch wissenschaftlich zu ergründen. Mitten aus dieser Arbeit hat ihn ein unerbitterliches Geschick herausgerissen. Seine äusserst gewinnende Persönlichkeit, seine Leutseligkeit, seine so gütige Wesensart und Hilfsbereitschaft gegenüber jedermann hat Köck eine grosse Zahl von dankbaren Schülern und Freunden geschaffen. Aber auch unter den engeren Fachkollegen des In-und Auslandes sowie unter seinen Berufskollegen auf der Hochschule erfreute er sich einer besoderen Wertschätzung, was in seiner seinerzeitigen Wahl zum Rektor der Hochschule für Bodenkultur für das Studienjahr 1935/36 sichtlichen Ausdruck fand. Wenn auch Köck infolge der ihm eigenen Bescheidenheit in aller Stille seinen studien und Arbeiten um die Weiterentwicklung des Pflanzenschutzes oblag, so können wir doch heute an den Ergebnissen und Erfolgen seiner emsigen Arbeit die Unvergänglichkeit seiner Verdienste ermessen, die er sich um den Ausbau und die Förderung des Pflanzenschutzes in wissenschaftlicher und praktischer Hinsicht in der Ostmark und darüber hinaus erworben hat.

HOCHSCHULE FÜR BODENKULTUR, WIEN XVIII–110, GREGOR MENDELSTR. 33.

VERÖFFENTLICHUNGEN

Bücher

1. Pflanzenschutz, 3 Bände (Feldbau, Obst-und Weinbau, Gemüse-und Gartenbau) von Köck-Fulmek, Verlag Carl Gerolds Sohn, Wien.

2. Schädlinge im Obstbau und ihre Bekämpfung, Köck-Löschnig-Miestinger, Scholle-Verlag, Wien.
3. Pflanzenschutz im Klein-und Siedlergarten, Köck, Scholle-Verlag, Wien.

Publikationen

1. Die wichtigsten pilzparasi-tären Erkrankungen unserer gebräuchlichsten Handelspflanzen und ihre Bekämpfung. Landes-Amstblatt des Erzherzogtumes österreich unter der Enns, Nr. 24/1910 und Nr. 1/1911.
2. Die häufigsten pilzlichen Krankheiten unserer wichtigsten Nadelhölzer und ihre Bekämpfung. Landes-Amtsblatt des Erzherzogtumes österreich unter der Enns, Nr. 12 und 13, 1908.
3. Die wichtigsten Krankheiten und Schädlinge unserer gebräuchlichsten Ziersträucher und Zierpflanzen und ihre Bekämpfung. Landes-Amtsblatt des Erzherzogtumes österreich unter der Enns, Nr. 23 und 24/1907, Nr. 2, 3, 4, 1908.
4. Die wichtigsten pilzlichen Krankheiten unserer Laubbäume und ihre Bekämpfung. Landes-Amtsblatt des Erzherzogtumes österreich unter der Enns, Nr. 4, 5/1909.
5. Beiträge zum Studium der Blattrollkrankheit. Monatschefte f. Landwirtschaft, 1910.
6. Unsere gegenwärtigen Kenntnisse über die Blattrollkrankheit der Kartoffel. Monatshefte f. Landwirtschaft, 1909.
7. Kartoffelschorf und Kartoffelkrebs. Mitteilungen d. Verb. landw. Versuchsstationen in österreich, Nr. 20.
8. Die wichtigsten Kartoffelkrankheiten und ihre Erkennung auf dem Felde. Monatshefte f. Landwirtschaft, 1913.
9. Die wichtigsten Krankheiten und Schädlinge der Futterpflanzen und ihre Bekämpfung. Monatshefte f. Landwirtschaft, 1908.
10. Der amerikanische Stachelbeermehltau. Monatshefte f. Landwirtschaft, 1908.
11. Der nordamerikanische Stachelbeermehltau und seine Bekämpfung. Der Obstzüchter, Nr. 8, 1913.
12. Ergebnisse mit der Iszleibschen Nährsalzimprägnation. östrerr. Ungar. Zeitschrft. für Zuckerindustrie und Landwirtschaft, II. Heft, 1906.
13. Über Cotyledonarknospen dicotyler Pflanzen. Österr. Botan. Zeitschrft., 1908, Nr. 2.
14. Vergleichende Bodentemperaturmessungen. Zeitschrift f. d. landw. Versuchswesen in österreich, 1918, S. 596.
15. Ein neuer Hefetriebskraftapparat. Zeitschrift. f. d. landw. Versuchswesen in österreich, 1906.
16. Beitrag zur Kenntnis der Verbreitung und der Bekämpfung des falschen Mehltaues der Gurken. Zeitschrft. f. d. landw. Versuchswesen in österreich, 1908, S. 128.
17. Über das Auftreten des nordamerikanischen Stachelbeermehltaues und des Eichenmehltaues in Galizien. Zeitschrft. f. Pflanzenkrankheiten, XX. Bd., 8. Heft, 1910.
18. Der Eichenmehltau, seine Verbreitung in österreich-Ungarn und seine Bedeutung in forstlicher Beziehung. Zeitschrft. f. d. landw. Versuchswesen in österreich, 1910.
19. Ein für österreich neuer Rosenschädling. Zeitschrft. f. d. landw. Versuchswesen in österreich, 1905.
20. Schorf, Monilia und Weissfleckenkrankheit auf verschiedenen Obstsorten. Zeitschrft. f. d. landw. Versuchswesen in österreich, 1911, S. 209.
21. *Septoria Lycopersici* auf Paradeispflanzen und *Phyllosticta Cyclaminis* auf *Cyclamen persicum.* Zeitschrft. f. d. landw. Versuchswesen in österreich, 1905.
22. Die wichtigsten Schädlinge der land-und fortswirtschaftlichen Kulturpflanzen und ihre Bekämpfung. Vademekum für den Landwirt, 11. Auflage.
23. Bericht über die von der k. k. Pflanzenschutzstation im Jahre 1911 durchgeführten Versuche zum Studium der Blattrollkrankheit der Kartoffel. Zeitschrft. f. d. landw. Versuchswesen in österreich, 1912, S. 179.
24. Ergebnisse der im Jahre 1912 durchgeführten Versuche und Untersuchungen über die Blattrollkrankheit der Kartoffel. Zeitschrft. f. d. landw. Versuchswesen in österreich, 1913, S. 89.
25. Studien über die Blattrollkrankheit der Kartoffel. Zeitschrft. f. d. landw. Versuchswesen in österreich, 1914, Heft. 5.
26. Die Schwarzbeinigkeit der Kartoffeln, ihre Ursache, wirtschaftliche Beseutung und Bekämpfung. österr. Zeitschrft. f. Kartoffelbau, Folge 1, Nr. 9.
27. Blausäurebegasungsversuche an Kartoffelpflanzgut. Fortschritte der Landw. Jahrg. 8, Heft 5, S. 102.
28. Die Bedeutung der kulturellen Bekämpfungsmethoden im praktischen Pflanzenschutz. Zeitschrft. f. Pflanzenkrankh. und Pflanzenschutz, Bd. 42, S. 383, 1932.
29. Über die Bedeutung der biologischen Bekämpfungsmethoden für den praktischen Pflanzenschutz. Zeitschrft. f. Pflanzenkrankh. und Pflanzenschutz, Jahrg. 1933, Bd. 43, S. 358.

30. Essig als Saatgutbeizmittel? Fortschritte d. Landw., Jahrg. 7, S. 226.
31. Unkrautbekämpfung mit Schwefelsäure. Die Landeskultur, Nr. 10, 1935.
32. Der Schwefeldioxydgehalt der Luft und sein Einfluss auf die Pflanze. Fortschritte d. Landw., Jahrg. 4, S. 170, 1929.
33. Einiges über die Frostwirkungen des Winters 1928/29 bei unseren Obstbäumen. Gartenzeitung der österr. Gartenbaugesellschaft, 1930.
34. Über die Bedeutung des Uspuluns als Pflanzenschutzmittel, speziell als Saatgutbeizmittel. Zeitschrft. f. d. landw. Versuchswesen in Österreich, 1919, S. 257.
35. Beiträge zum Problem der Schorfkrankheiten unseres Kernobstes mit besonderer Berücksichtigung der Widerstandsfähigkeit einzelner Sorten. Die Landwirtschaft, 1927, S. 264, 307, 346.
36. Über das Verhalten der verschiedenen Kartoffelsorten gegenüber dem Erreger der Kartoffelknollenfäule (*Phytophthora infestans*). Fortschritte d. Landw. Jahrg. 6, S. 518, 1931.
37. Über das Verhalten der einzelnen Apfelsorten gegenüber dem Apfelmehltau. Fortschritte d. Landw. Jahrg. 1927, Heft 18.
38. Der gärtnerische Pflanzenschutz in Österreich. Festschrift d. österr. Gartenbaugesellschaft, 1827–1927.
39. Das Wesen der Gelbrostbekämpfung des Weizens. Fortschritte d. Landw. Jahrg., 1927, Heft 10.
40. Lohnt sich ein weiterer Ausbau der chemischen Methoden im praktischen Pflanzenschutz? Zeitschrft. f. Pflanzenkrankh. und Pflanzenschutz, Bd. 42, S. 233, 1932.
41. Über die Rauchgasfiltrationswirkung eines Fichtenbestandes. Phytopathologische Zeitschrft., Bd. VII, S. 255, 1934.
42. Ludwig Hecke zum Gedächnis. Österr. Botan. Zeitschrft., Bd. 84, S. 295, 1935.

CONCENTRATION AND CHARACTERIZATION OF THE EMETIC PRINCIPLE PRESENT IN BARLEY INFECTED WITH GIBBERELLA SAUBINETII[1]

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The toxicity of scabby grain to man and certain animals has been well established. The first reports (5, 6, 12) concerned the toxicity of flour made from diseased grain. People eating bread baked from this flour became sick and emesis resulted. Later, feeding experiments (1, 2, 4, 8, 10, 11, 13, 16, 17, 21) demonstrated that scabby barley was toxic and induced emesis in certain animals, especially those with simple stomachs.

Although the toxicity of scabby barley to certain animals has been well established, the published information concerning the nature of the emetic principle present in grain infected with *Gibberella saubinetii* (Mont.) Sacc. and *Fusarium* species is limited and conflicting. Pomaskii (14, 15), who was the first to offer any evidence as to the nature of the toxin, considered that it was probably a nitrogenous glucoside. Danckwortt's (3) investigation offered no definite evidence as to the nature of the toxin. His tests for alkaloids proved negative. Popp (16) attributed the formation of the toxic principle to the decomposition of protein compounds from which toxalbumins or similar toxic nitrogenous compounds were formed. The investigation of Schroeter and Strassberger (18) led them to believe that choline or easily hydrolyzable choline fatty-acid esters produced the injurious action. Dickson, Link, Roche, and Dickson (4) concluded that the active substance was associated with the fractions containing glucosides or basic nitrogen compounds.

The present investigation was undertaken to learn something more of the characteristics of the emetic principle.

MATERIAL AND METHODS

In the summer of 1938, observations on a number of barley fields in the vicinity of Ames, Iowa, from the latter part of June until harvest disclosed a general epiphytotic of scabby barley. A field of Ioglos barley on the farm of the Department of Agricultural Engineering, Iowa State College, was severely infected with *Gibberella saubinetii*. Preliminary tests of aqueous extracts of this barley on swine indicated that the diseased grain contained the emetic principle. In order to obtain barley more heavily infected than the ordinary field run, arrangements were made with the Department of Agricultural Engineering to obtain the screenings after the barley was cleaned. Screenings weighing, respectively, 10, 15, 24, 27, and 32 lb. per bu. were used throughout the investigation. The screenings weighing 10 and 15 lb. per bu. contained much plant refuse, and recleaning was necessary. Germination and isolation experiments with the screenings indicated an inverse relationship between the weight of the grain and the prevalence of the pathogen. Table 1 shows the percentage of infected grain for the respective bushel weights.

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[1] From a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree of doctor of philosophy.

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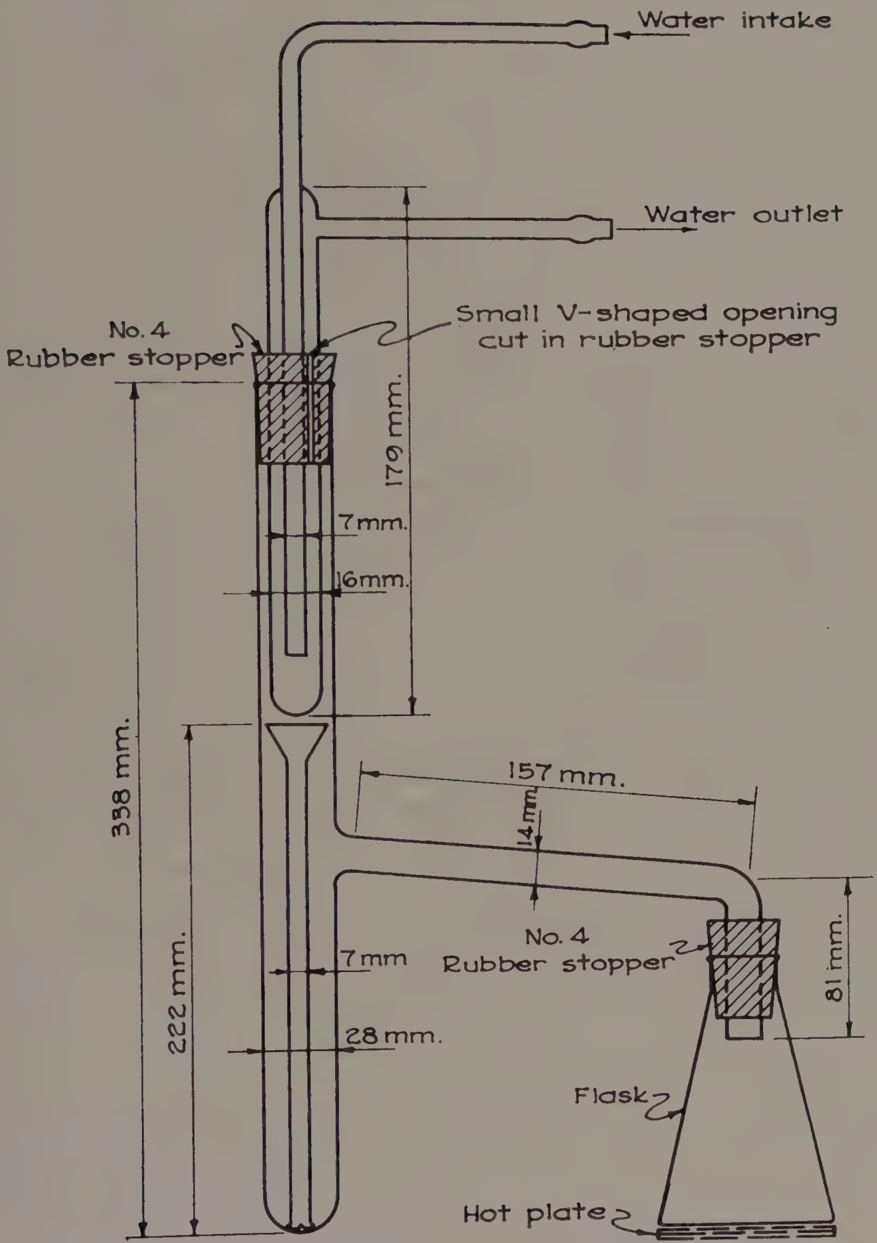
TABLE 1.—*Percentage of grain infected with Gibberella saubinetii in barley of different weights per bushel*

Bushel weights in pounds	10	15	24	27	32
Percentage infected	95	94	90	90	87

Since swine are susceptible to the emetic principle present in scabby barley, they were chosen as the assay animals to test for the presence of the toxin in various extracts and concentrates. During the course of the investigation, the extracts and concentrates were administered by a stomach tube to 16 swine of various breeds. The pigs were obtained when weighing not less than 25 lb. nor over 50, and retained until each reached a weight of approximately 150 lb. Although the heavier pigs were still susceptible to the emetic principle, they became more difficult to handle and were, therefore, discarded. After administering the test solutions, the swine were observed until emesis had subsided. In only a few cases was this period prolonged more than 1 hour.

The emetic principle was removed from the whole grain by placing a weighed quantity of the scabby barley in beakers and adding twice its weight in distilled water. The beakers were covered with watch glasses and placed on an electric plate for 12 hours. The solution temperature was maintained at approximately 92° C. The aqueous extracts were then decanted, the barley washed once with distilled water, and the washings added to the first aqueous extracts. The extracts were placed in beakers and evaporated nearly to dryness on a water bath. A brown residue remained. Warm methyl alcohol was thoroughly mixed with the residue and the material filtered with suction. The filtrate was placed in an evaporating dish and put on a steam bath to remove the methyl alcohol. After most of the methyl alcohol had been removed, a small quantity of distilled water was added to the concentrated methyl alcohol-soluble material and the evaporation of the methyl alcohol was completed. A brown residue remained that was dissolved in a small quantity of dilute sodium hydroxide solution and placed in the extractor shown in figure 1. Extraction with ether for 24 hours was



Water intake
Water outlet
Small V-shaped opening cut in rubber stopper
No. 4 Rubber stopper
179 mm.
7mm.
16mm.
338 mm.
222 mm.
157 mm.
14mm.
7mm
28 mm.
No. 4 Rubber stopper
81 mm.
Flask
Hot plate

FIG. 1. Extractor with condenser used for extracting small volumes.

sufficient to remove the emetic principle from the alkaline solution. Anhydrous sodium sulphate was added to the ether soluble portion and the material was allowed to stand overnight or longer before the ether-soluble material was poured on a watch glass. After the ether was evaporated, a very small quantity of yellow, syrupy material remained.

Solubility behavior, physical examination, classification tests, and an analysis for certain elements were carried out with the yellow concentrate. Attempts were made to crystallize, to isolate as a hydrochloride salt, and to prepare various derivatives of the yellow concentrate. After obtaining doubtful alkaloid tests with the yellow concentrate, it was dissolved in a chloroform-ether mixture and extracted with a 2 per cent HCl acid solution in order to bring down any complex organic bases. The acid-soluble portion was then tested with several alkaloid reagents.

EXPERIMENTAL RESULTS

Toxicity of the Aqueous Extracts

Previous investigations (1, 2, 4, 16, 18) have shown that the emetic principle present in scabby barley was soluble in water. In order to confirm this earlier work, a number of aqueous extracts were tested for their potency when different quantities of barley were extracted at different temperatures for various lengths of time. Table 2 summarizes these data. The results indicate that the aqueous extracts contained the emetic principle. In only one instance did a test animal fail to respond and that was when the aqueous extract obtained from only 20 g. of barley was administered.

TABLE 2.—*Toxicity of the aqueous solutions extracted from different quantities of scabby barley at different temperatures for various lengths of time*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*	*Minutes*	*Number*
2	20	15	20–25	24	......	0
1	100	15	20–25	24	12	9
2	100	15	20–25	24	24	15
1	100	15	7.2	48	16	5
2	300	32	7.2	168	70	8
15	240	27	92.0	12	19	9
16	240	27	92.0	12	19	4

Test animals 1, 2, 15, and 16 were each given the aqueous extract obtained from 420 g. of barley known to be free from *Gibberella saubinetii.* In no instance was there any evidence of emesis. From these results it was assumed that an aqueous extract from healthy barley was not the cause of emesis in swine.

Effect of Acidifying Aqueous Extracts

The stability of the emetic principle in the presence of acid was tested by adding hydrochloric acid to the aqueous extracts before administering them to the animals. The results obtained from this treatment are given in the following table.

These results may be evaluated by comparing the number of vomitions of pigs 15 and 16 in tables 2 and 3. In each case the extraction time

TABLE 3.—*Effect of acidifying aqueous extracts before administering them to swine*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Acidity of aqueous extract	Acidity after adding HCl	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*	*pH*	*pH*	*Minutes*	*Number*
16	240	27	92	12	5.82	3.36	19	6
15	240	27	92	12	5.65	2.26	15	4

was 12 hr. at 92° C. from 240 g. of scabby barley weighing 27 lb. per bu. Pig no. 16 vomited more when given the acidic extract, while less emesis resulted when such an extract was given to pig no. 15. These two trials indicate that the emetic principle was not inactivated when the aqueous extracts had pH values of 3.36 and 2.26.

Effect of Autoclaving Aqueous Extracts

To corroborate previous reports (1, 2) that the emetic principle is thermostable, aqueous extracts were autoclaved at a pressure of 15 lb. for 25 min. before their dispensation to swine.

TABLE 4.—*Effect of autoclaving aqueous extracts at 15 pounds' presuure for 25 minutes before administering them to swine*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Acidity of aqueous extract	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*	*pH*	*Minutes*	*Number*
1	100	15	7.2	48	....	28	4
16	240	27	92.0	12	5.90	......	[a]
15	240	27	92.0	12	5.65	......	0
16	240	27	92.0	12	5.74	22	1

[a] No emesis, but appeared sick and refused feed.

The results indicate that autoclaving aqueous extracts reduces the potency of the emetic principle. Pig no. 15 failed to react, but pigs 1 and 16 either vomited or appeared sick. A comparison of the reaction of pig no. 16, when given an acidified aqueous extract and an autoclaved aqueous extract, may be made by comparing the results recorded in tables 3 and 4. In each case the aqueous extracts were made from 240 g. of 27 lb. per bu. barley. Only the autoclaved extracts reduced the amount of emesis. It may be assumed, therefore, that autoclaving partly inactivates the emetic principle.

Effect of Autoclaving Acidified Aqueous Extracts

The reduction in potency of the toxic substance obtained from autoclaving aqueous extracts suggested autoclaving acidified aqueous extracts. Table 5 shows the results obtained from autoclaving the extracts after the latter had been adjusted to low pH values by the addition of hydrochloric acid.

TABLE 5.—*Effect of autoclaving acidified aqueous extracts of various pH values at 15 pounds' pressure for 25 minutes before administering them to swine*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Aqueous extract	After addition of HCl	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	*°C.*	*Hours*	*pH*	*pH*	*Minutes*	*Number*
16	240	27	92	12	5.86	3.25	18	2
15	240	27	92	12	5.86	2.06	......	0
16	240	27	92	12	5.70	1.96	......	0
15	240	27	92	12	5.97	1.95	43	1
15	240	27	92	12	5.74	1.93	24	3
16	240	27	92	12	5.95	1.82	......	0
15	240	27	92	12	5.95	1.80	......	0

A comparison of the number of vomitions in tables 4 and 5 substantiates the conclusion that the addition of hydrochloric acid to an aqueous extract before autoclaving had little, if any, effect. The data in both tables indicate a partial inactivation of the emetic principle. Since the addition of hydrochloric acid before autoclaving does not reduce the potency of the emetic principle further than the autoclaving of an aqueous extract, it is doubtful if the acid has any effect, in spite of the favorable conditions for hydrolysis.

Effect of Adding Alkali to Aqueous Extracts

Beller and Wedemann (1) have suggested that scabby barley may be utilized as feed for swine if the barley be boiled for 1 hour and followed with washing or neutralization of the toxic extract with sodium hydroxide. Several attempts were, therefore, made to confirm this earlier work. Various amounts of sodium hydroxide solution were added to the extracts in order to obtain a succession of pH values ranging from 7.15 to 11.65.

Pig no. 16 vomited 4 times when given an aqueous extract, pH 7.15. The same number of vomitions (Table 2) was obtained from this assay animal when given an aqueous extract of the same strength. When the extracts were made more alkaline and given to the same pig, the results (Table 6) indicate a reduction in the activity of the emetic principle. Pig no. 15 vomited twice when the extracts were at pH 7.97 and pH 10.15, but only appeared sick when administered extracts having pH values of 11.00 and 11.65.

Effect of Evaporating Aqueous Extracts to Dryness

Thermostability data (Table 2) suggested a possible means of concen-

TABLE 6.—*Effect of alkalizing aqueous extracts from scabby barley before administering them to swine*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Aqueous extract	After addition of NaOH	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*	*pH*	*pH*	*Minutes*	*Number*
16	240	27	92	12	5.88	7.15	27	4
15	240	27	92	12	5.94	7.97	25	2
16	240	27	92	12	5.60	9.06	31	2
15	240	27	92	12	5.85	10.15	30	2
15	240	27	92	12	5.65	11.00	......	[a]
16	240	27	92	12	5.85	11.53	43	2
16	240	27	92	12	5.56	11.56	......	0
15	240	27	92	12	5.75	11.65	......	[a]

[a] No emesis, but appeared sick and refused feed.

trating the aqueous extract without altering the chemical nature of the emetic principle. The aqueous extract was divided equally among 8 beakers and evaporated to dryness on a water bath. As shown in table 7, the dried extracts were redissolved in water the same day they were obtained, or were allowed to remain as long as 7 days in the dried state.

TABLE 7.—*Effect of evaporating aqueous extracts from scabby barley to dryness and redissolving them in water before administering to swine*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extration period	Time evaporated extracts remained in dried state	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*	*Days*	*Minutes*	*Number*
7	250	32	7.2	24	1	22	11
7	250	32	7.2	24	7	21	11
8	250	24	7.2	24	0	23	9
4	500	32	7.2	24	0	28	6
3	500	32	7.2	24	0	18	9

The data in table 7 indicate that the redissolved dried extracts had not lost their ability to produce emesis in swine. The one dried extract, which remained as such for 7 days before redissolving and administering, was as effective in producing emesis as any dried extract tested.

Toxicity of Filtrates Obtained from Filtering Concentrated Aqueous Extracts, and Gummy Residues Remaining on Filter Papers and Sides of Beakers

During the removal of water and certain volatile substances from the aqueous extracts by evaporation over a water bath, a brown gummy residue formed on the sides of the beakers as well as in the concentrated extracts. In order to determine whether this gummy material was toxic, the concentrated extracts were filtered with suction before being evaporated to dryness. Tests were made of the water-soluble material contained in the filtrates, and the gummy residues remaining on the filter papers and sides of the beakers. The results are summarized in table 8.

TABLE 8.—*Toxicity of the filtrates obtained from filtering concentrated aqueous extracts, and of the gummy residues remaining on the filter papers and sides of the beakers*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Nature of material administered	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*		*Minutes*	*Number*
2	800	32	7.2	216	Filtrate	24	18
3	800	32	7.2	216	Gummy residue[a]	22	5
6	500	32	7.2	24	Filtrate	17	7
4	500	32	7.2	24	Gummy residue[b]	......	0

[a] The gummy residue was not washed on the filter paper.
[b] The gummy residue was given a thorough washing with water.

The responses of pigs nos. 2 and 6 indicate that the emetic principle passed into the filtrates. Pig no. 3 vomited 5 times when given the gummy residue that was not washed when the concentrated aqueous extract was filtered. The negative response of pig no. 4 indicates that a thoroughly washed gummy residue was not toxic.

Vacuum, Steam, and Fractional Distillation

Purification by vacuum distillation was attempted 3 times, but the foamy nature of the aqueous extracts made it impossible to use this method. The results obtained from subjecting concentrated aqueous extracts to steam distillation are presented in table 9.

TABLE 9.—*Toxicity of the distillate and residues resulting from steam distillation of concentrated aqueous extracts obtained from scabby barley*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Nature of material administered	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*		*Minutes*	*Number*
3	100	15	7.2	52	Distillate	......	0
1	100	15	7.2	52	Residue	25	13
3	100	15	7.2	105	Residue	13	11
2	100	32	7.2	23	Residue	32	7

The results in table 9 show that the emetic principle remained in the residue. Pig no. 3, which received the distillate, showed no symptoms of the champing that generally preceded emesis. It continued eating throughout the observation period.

A further attempt to separate the emetic principle from the aqueous extracts was made by concentrating the extracts to a small volume and fractionally distilling. Table 10 gives the different fractions tested and the results obtained.

TABLE 10.—*Toxicity of the distillates and residues resulting from the fractional distillation of concentrated aqueous extracts*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction period	Fractions administered	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	*°C.*	*Hours*		*Minutes*	*Number*
4	500	32	7.2	24	100–113[a]	......	0
5	....	......	......	......	113–130	......	0
3	....	......	......	......	Residue	18	9
5	500	32	7.2	24	100–130	......	0
3	....	......	......	......	130–170	......	0
4	....	......	......	......	Residue	28	6

[a] Distillation temperature at which the fraction was obtained.

No vomitions were obtained when the distillates were administered to the test animals. The residues, however, proved toxic. The failure of a distillation temperature of 170° C. to inactivate the emetic principle is rather significant.

Methyl and Ethyl Alcohols as Differential Solvents

After establishing the fact that the aqueous extracts could be taken to dryness (Table 7), without a loss in the potency of the emetic principle, further methods were attempted to free the toxin from accompanying materials. The use of methyl and ethyl alcohols as differential solvents proved satisfactory in eliminating a large portion of the inactive material. To simplify the alcohol extractions, the aqueous extracts were removed from the water bath before being evaporated to dryness and the alcohol added and warmed. It was necessary to use a spatula to free any residue adhering to the sides and bottoms of the beakers before the alcohol could be thoroughly mixed with the concentrated material. The alcohol-soluble material was filtered with suction and the insoluble material washed twice with alcohol. The data given in table 11 show the efficiency of methyl alcohol as a solvent for the further purification of the toxic substance.

The results recorded in table 11 indicate that the methyl-alcohol-insoluble material was not toxic to swine. In one case, pig no. 14, a response was obtained from dispensing the methyl-alcohol-insoluble material. This

TABLE 11.—*Toxicity of the methyl-alcohol-soluble and insoluble portions obtained from extracting the residues remaining after the aqueous extracts were evaporated nearly to dryness*

Test animal	Wt. of scabby barley used in extracting toxin	Weight of barley per bushel	Temperature of solution during extraction	Duration of extraction	Kind of material administered	Interval between administering and vomition	Vomitions
No.	*Grams*	*Pounds*	°*C.*	*Hours*		*Minutes*	*Number*
9	250	24	7.2	24	Soluble	23	9
10	....	......	....	......	Insoluble	......	0
10	250	24	7.2	24	Soluble	43	7
8	....	......	....	......	Insoluble	......	0
12	160	24	7.2	24	Soluble	14	4
11	....	......	....	......	Insoluble	......	0
13	1200	27	7.2	12	Insoluble	......	0
14	180	10	100.0	1	Soluble	12	26
14	720	10	100.0	1	Insoluble	32	4[a]
12	400	27	100.0	1	Soluble	23	4

[a] Insoluble material not washed with methyl alcohol when filtered.

response may be explained by the fact that the insoluble material was not washed with methyl alcohol when filtered with suction.

Beller and Wedemann (1) simplified their extraction method by placing the scabby barley in boiling water for 1 hour. The evidence presented in table 11 is the first instance of an attempt to test their recommendation. In 3 instances the barley was extracted with water at 100° C. The material resulting from these extractions was given to two different test animals (pigs nos. 12 and 14) and emesis was produced in each case. The amount of emesis from pig no. 14 was greater than that observed from any test animal throughout the course of the investigation.

Although ethyl alcohol proved to be a differential solvent for removing the emetic principle from the concentrated aqueous extracts, it was abandoned in favor of methyl alcohol. When ethyl alcohol was used, the insoluble portion had a tendency to remain in a gummy state and this made the separation more difficult.

Precautions were always taken to remove the methyl alcohol from any of the test solutions, but it was entirely possible that traces of this alcohol remained. A 5 per cent solution of methyl alcohol was administered to 3 different pigs to determine whether emesis could be produced. In no instance was there any evidence of the symptoms accompanying emesis. Beller and Wedemann (1) administered 1, 5 and 7 per cent solutions of ethyl alcohol to swine and observed no emesis.

Ether as a Differential Solvent

In order to eliminate any foreign material soluble in water and methyl alcohol, ether was used as a further differential solvent to extract the methyl-alcohol-soluble material. The methyl alcohol was removed over a water bath and the residue was dissolved in a small quantity of distilled water and extracted with a separatory funnel or the continuous extractor (Fig. 1). The results from several extractions are given in table 12.

TABLE 12.—*Toxicity of the ether-soluble and insoluble portions obtained from extracting aqueous solutions of the methyl-alcohol-soluble material*

Test animal	Wt. of scabby barley used in extracting toxin	Method of ether extraction	Ether extractions	Duration of ether extraction	Kind of material administered	Weight of ether-soluble material	Interval between administering and vomition	Vomitions
No.	*Grams*		*Number*	*Hours*		*Grams*	*Minutes*	*Number*
11	250	Separatory funnel	10	......	Soluble	......	14	5
12	......	......	......	......	Insoluble	......	......	0
12	300	Separatory funnel	10	......	Soluble	......	16	9
11	......	......	......	......	Insoluble	......	19	11
11	300	Separatory funnel	15	......	Insoluble	......	22	9
11	300	Continuous	......	12	Soluble	0.274	15	6
12	......	......	......	......	Insoluble	......	21	3
12	300	Continuous	......	24	Insoluble	......	......	0
12	300	Continuous	......	36	Insoluble	......	......	0
14	180	Continuous	......	24	Soluble	0.537	14	18
14	......	......	......	......	Insoluble	......	......	0

[a] Barley of various bushel weights from which toxin was extracted with water at 92° C. for 12 hours.

The results recorded in table 12 show that the emetic principle was present in the ether-soluble and insoluble portions. Fifteen extractions with the separatory funnel proved insufficient to remove completely the toxin from the aqueous portion. The results obtained with the continuous extractor were more promising. Twelve hours of continuous extraction did not suffice to remove completely the emetic principle, but extractions of 24 and 36 hours proved satisfactory. From these results the assumption may be made that the emetic principle is not very soluble in ether. After evaporating the ether, only a small amount of a yellow, syrupy material remained. Table 12 indicates the weights of this material on two occasions. Test animal no. 14 vomited 18 times when given 0.537 g. of the yellow concentrated material.

Ether Extractions from Basic and Acidic Aqueous Solutions

The promising results shown in table 12 suggested further methods for purification, as well as an opportunity to learn something of the chemical nature of the toxic substance. The acid reaction of every aqueous extract, the inactivation of the toxin with sodium hydroxide, as reported by Beller and Wedemann (1), but not confirmed in this investigation, and the solubility of the concentrated methyl-alcohol-soluble material in dilute sodium hydroxide solution, all suggested that the unknown might have one or more acidic groups strong enough to react with sodium hydroxide and thus be insoluble in ether. An opportunity to determine whether the toxin would form an ether-insoluble hydrochloride salt was presented by attempting to extract an acidified aqueous solution of the concentrated methyl alcohol-soluble material with ether. Data obtained from several extractions are summarized in table 13. The results indicate that the emetic principle could be extracted from either basic or acidic aqueous solutions. Evidently, the unknown was not strongly acidic nor had it the property of forming a hydrochloride salt. One significant comparison was the amount of ether-soluble material obtained from the basic and acidic extractions. From two acidic extractions 0.720 and 0.750 grams of the yellow concentrate were obtained. The largest amount of ether-soluble material obtained from a basic extraction was 0.173 g. This reduced amount of ether-soluble material was still as potent in affecting emesis as the larger amount obtained from the acidic extractions. Test animal no. 16 vomited 3 times after being administered 0.077 g. of the yellow concentrate. Ether extractions from basic solutions tended to eliminate approximately half a gram of foreign material which was ordinarily included in the ether-soluble material extracted from an acidic solution.

TABLE 13—*Toxicity of the ether-soluble and insoluble portions obtained from extracting basic and acidic aqueous solutions of the methyl-alcohol-soluble material*[a]

Test animal	Acid or base added	Solution extracted from	Times extracted with ether	Duration of continuous ether extraction	Kind of material administered	Weight of ether soluble portion	Interval between administering and vomition	Vomitions
No.		*pH*		*Hours*		*Grams*	*Minutes*	*Number*
12	5 ml. HCl[b]	....	15	......	Soluble	....	15	7
11	....	....	......	......	Insoluble	....	......	0
11	5 ml. NaOH[c]	....	15	......	Soluble	....	23	6
12	....	....	......	......	Insoluble	....	......	0
12	5 ml. HCl[b]	....	......	36	Soluble	0.720	18	8
11	....	....	......	......	Insoluble	....	......	0
12	10 ml. NaOH[c]	....	......	12	Soluble	0.059	......	0
13	....	....	......	......	Insoluble	....	......	0
14	NaOH[d]	8.67	......	24	Soluble	0.173	9	18
14	HCl[e]	0.45	......	24	Soluble	0.750	25	8
14	NaOH[d]	8.60	......	24	Soluble	0.150	26	3
15	NaOH[d]	8.40	......	24	Soluble	0.114	16	2
16	NaOH[d]	11.35	......	24	Soluble	0.077	50	3
15	NaOH[d]	9.90	......	24	Soluble	0.125	33	2

[a] Original aqueous extracts made from scabby barley of various bushel weights at 92° C. for 12 hours.

[b] Concentrated HCl.

[c] Forty per cent NaOH.

[d] Dilute NaOH.

[e] Dilute HCl.

Attempts Leading to the Identification of the Emetic Principle

The emetic principle was collected by pouring the ether-soluble material on a watch glass. After the ether had evaporated the residue appeared as a homogeneous, yellow, syrupy material. The yellow material thickened somewhat on prolonged standing in a desiccator, but failed to harden after standing as long as 20 weeks. It had an unfamiliar odor, and prolonged smelling produced headache in man. Another characteristic was its slightly bitter taste.

On combustion the yellow concentrate burned slowly with a red flame. Carbon, but no inorganic residue, remained. An analysis for sulphur, nitrogen, chlorine, bromine, and iodine was made by using the sodium fusion method outlined by Kamm (7). The analysis for sulphur and the halogens was negative, but a positive test was obtained for nitrogen.

Although water and ether were used as solvents for the extraction and separation of the emetic principle, the yellow concentrate was sparingly soluble in these solvents. The concentrated material was insoluble in dilute hydrochloric acid, dilute sodium bicarbonate, benzene, petroleum ether, carbon tetrachloride, and carbon disulphide. It was soluble in methyl alcohol, ethyl alcohol, dilute sodium hydroxide, acetone, chloroform, and 1,4-dioxane. According to the scheme outlined by Shriner and Fuson (20), the solubility behavior indicates an acidic compound falling in the solubility class A_2. This class includes phenols, sulfonamides of primary amines, primary and secondary nitro compounds, imides and thiophenols. Since the yellow concentrate probably is not a pure substance, the solubility observations are of limited value.

The yellow concentrate was treated with a few classification reagents useful in detecting the presence of functional groups. Negative results were obtained when tests were made for the presence of phenolic and nitro-type compounds, for acidic hydrogens and for nonsaturation. A number of derivatives were attempted in order to learn more concerning the chemical behavior of the unknown, as well as to isolate it as a derivative. Several reagents, used to form derivatives of organic acids, phenolic type compounds, tertiary amines, and alkaloids, were employed, but no reactions took place, Attempts were made to precipitate the emetic principle as a hydrochloride salt, but negative results were obtained. Crytallization from a few solvents was unsuccessful. A further attempt to crystallize the yellow concentrate was made by placing it at −28° C. for 10 days, but no apparent change was observed.

The yellow concentrate was tested with such alkaloid reagents as Marquis', Mayer's, Hager's and Scheibler's. Marquis' reagent gave a wine-red coloration that turned brown upon the addition of more of the reagent. Mayer's, Hager's and Scheibler's reagents did not give a precipitate typical of a positive test for alkaloids, but produced only a cloudiness. Since these could not be considered as positive tests, an attempt was made to purify the material further and test for alkaloids again. The yellow concentrate was dissolved in a mixture of chloroform and ether and extracted with 2 per cent hydrochloric acid solution. The acid-soluble portion was then tested with various alkaloid reagents. This portion gave positive tests with Mayer's reagent, gold chloride, iodine, neutral lead acetate, and phosphotungstic acid. A negative test was obtained with platinum chloride. It appears from these results that the acid-soluble portion contains one or more complex nitrogen bases.

SUMMARY

Evidence has been presented indicating that the emetic principle present in barley infected with *Gibberella saubinetii* could be extracted from the whole grain, with water, over a wide temperature range.

Swine were used as the test animals to determine the presence or absence of the emetic principle in the extracts and concentrates. The extracts and concentrates were administered by means of a stomach tube.

The addition of hydrochloric acid to an aqueous extract failed to reduce the activity of the emetic principle. Autoclaving aqueous extracts or acidified aqueous extracts partly inactivated the emetic principle. Incomplete inactivation was obtained also by adding sodium hydroxide to aqueous extracts.

The emetic principle was not inactivated when aqueous extracts were evaporated to dryness.

Filtration of concentrated aqueous extracts indicated that the emetic principle passed into the filtrates.

Attempts to free the emetic principle from concentrated aqueous extracts by steam and fractional distillations were not successful. In all cases the toxic substance remained in the residues.

Methyl alcohol proved to be an efficient solvent for removing all the emetic principle from the concentrated aqueous extracts. Further purification of the methyl alcohol-soluble material was obtained by extracting concentrated aqueous solutions of the methyl alcohol-soluble material with ether. The emetic principle was soluble in ether when acid or alkali was added to concentrated aqueous solutions of the methyl alcohol-soluble material.

An analysis of the yellow, syrupy concentrate indicated the absence of sulphur and the halogens, and the presence of nitrogen.

The solubility tests, as well as the information obtained from the classification tests and attempted derivatives, were of limited value insofar as concerned the securing of information concerning the emetic principle, because the yellow concentrate probably was impure.

Alkaloid tests of the yellow concentrate were not too positive. In an attempt to eliminate additional foreign material, the yellow concentrate was dissolved in an ether-chloroform mixture and extracted with a 2 per cent hydrochloric acid solution. The acid-soluble portion gave positive alkaloid tests with Mayer's reagent, gold chloride, iodine, neutral lead acetate, and

phosphotungstic acid. A negative test was obtained with platinum chloride.

Although the results indicate that the emetic principle may be an alkaloid, the data are insufficient to warrant that conclusion.

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LITERATURE CITED

1. BELLER, K., and W. WEDEMANN. Untersuchung über die Schadwirkung amerikanischer Futtergerste (sog. Barley Federal Nr. II). Zeitsch. Infektionskrankheiten, parasitäre Krankheiten und Hygiene der Haustiere. 36: 103–129. 1929.
2. CHRISTENSEN, J. J., and H. C. H. KERNKAMP. Studies on the toxicity of blighted barley to swine. Minn. Agr. Exp. Stat. Tech. Bull. 113. 1936.
3. DANCKWORTT, P. W. Chemische Untersuchung der amerikanischen Giftgerste. Dtsch. Tierärztl. Wschr. 37: 170–171. 1929.
4. DICKSON, ALLAN D., KARL P. LINK, B. H. ROCHE, and JAMES G. DICKSON. Report on the emetic substances in Gibberella-infected barley. (Abstract) Phytopath. 20: 132. 1930.
5. DOUNIN, M. The Fusariosis of cereal crops in European Russia in 1923. Phytopath. 16: 305–308. 1926.
6. ERIKSSON, JAKOB. Fungoid diseases of agricultural plants. Baillière, Tindal, and Cox (London). 1912.
7. KAMM, OLIVER. Qualitative organic analysis. John Wiley & Sons, Inc. (New York). 1932.
8. MAINS, E. B., C. M. VESTAL, and P. B. CURTIS. Scab of small grains and feeding trouble in Indiana in 1928. Ind. Acad. Sci. Proc. 39: 101–110. 1930.
9. MIESSNER, H., and G. SCHOOP. Über den Pilzbefall amerikanischer ''Giftgerste.'' Deuts. Tierärztl. Wschr. 37: 167–170. 1929.
10. MUNDKUR, B. B. Some preliminary feeding experiments with scabby barley. Phytopath. 24: 1237–1243. 1934.
11. ———— and R. L. COCHRAN. Some feeding tests with scabby barley. (Abstract) Phytopath. 20: 132. 1930.
12. NAUMOV, N. A. Intoxicating bread. Min. Zeml. (Russia), Trudy Bi͡uro Miwol. i Fitopatol., Uchen. Kom., (12) p. 216. 1916. (Original not seen.) Abstracted in Exp. Stat. Rec. 36: 747. 1917.
13. OPPERMANN and DOENECKE. Fütterungsversuche mit amerikanischer ''Giftgerste''. Deuts. Tierärztl. Wschr. 37: 165–167. 1929.
14. POMASKII, A. Regarding the changes in chemical composition of rye resulting from the activity of certain *Fusarium* forms. Mat. Mikol. i Fitopatol. Ross., 1: 77–106. 1915. (Original not seen.) Abstracted in Exp. Stat. Rec. 35: 845. 1916.
15. ————. Changes in the chemical composition of rye under the influence of species of Fusarium. Soobshch. Bi͡uro Chastn. Rast., (Petrogad), 3: 32. 1916. (Original not seen.) Abstracted in Exp. Stat. Rec. 36: 633. 1917.
16. POPP, M. Untersuchung über die amerikanische Giftgerste. Chem. Zeit. 54: 715. 1930.
17. ROCHE, B. H., G. BOHSTEDT, and JAMES G. DICKSON. Feeding scab-infected barley. (Abstract) Phytopath. 20: 132. 1930.
18. SCHROETER, G., and L. STRASSBERGER. Cholin als Schadstoff in kranker Gerste. Biochem. Zeit. 232: 452–458. 1931.
19. SHANDS, R. G. Longevity of *Gibberella saubinetii* and other fungi in barley kernels and its relation to the emetic effect. Phytopath. 27: 749–762.
20. SHRINER, RALPH L., and REYNOLD C. FUSON. The systematic identification of organic compounds. John Wiley & Sons, Inc. (New York). 1935.
21. TITUS, HARRY W., and A. B. GODFREY. Comparison of scabbed barley, normal barley, and yellow corn in diets for laying chickens. U. S. Dept. Agr. Tech. Bull. 435. 1934.

TRANSMISSION OF DIAMOND CANKER OF THE FRENCH PRUNE

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In diamond canker parts of the trunk and branches of the French-prune tree (*Prunus domestica* var. Agen), become greatly thickened in a characteristic manner,[1] and diamond or oval-shape excrescences appear on the main branches, especially at pruning cuts (Fig. 1, A), extending upward and suggesting a systemic spread of the disease in the tree.

Localized swellings or cankers also may appear on the secondary branches, on trees that may or may not show the disease lower down (Fig. 1, C).

A cross section (Fig. 1, B) shows that these thickenings are composed of hard, leathery, cork tissue of the bark.

Badly affected trees have a tendency to send up sprouts from the body; on some of these sprouts, which originate directly from diseased bark, a roughening and scurfiness with pimply excrescences is seen (Fig. 1, D) extending, in some cases, almost to the end of the current season's growth. A cross section of such a young stem (Fig. 2, A) shows that the roughening is caused by the successive formation and pushing out of layers of phellogen and cork cells, much as in the normal bark formation of rough-bark, woody stems. The appearance of these bark symptoms on a rapidly developing shoot may keep pace with its growth for as much as 6 or 8 feet in a single season, although other shoots on the same or other trees similarly affected in the trunk and body may not show them at all. As sprouts that show the bark symptoms grow older, if they are left on the tree for several years, the basal portion (Fig. 1, F) develops the typical diamond-canker condition. The bark-roughening symptoms tend to concentrate or become more pronounced at the end of the growth of each season (Fig. 1, E).

No leaf symptoms have been detected in this disease.

Many trees show only a few cankers, these being located here and there on the sides of the main branches, usually at pruning cuts (Fig. 2, B), and most frequently on the south side. These may or may not develop further in the tree or branch, in the seemingly systemic manner mentioned above.

Instances are not uncommon where the appearance of diamond canker commences in wound-healing tissues, as in a pruning cut (Fig. 2, B), a bruise (Fig. 2, D), or a lesion produced by bacterial gummosis, caused by *Phytomonas cerasi* (Fig. 3, A). Typical cankers also are seen rather frequently on French prune in top-grafted trees, whether this variety composes the stock or the top. If the reciprocal portion of the tree is of any other species or variety (myrobolan, peach, apricot, or any variety of plum, for instance), the canker symptoms end abruptly at the union. Trees are occasionally observed where both stock and top are of French prune; these being trees of the ordinary type that have been worked over to supposedly superior strains. Trees of this sort often show diamond canker starting at the union, either in the stock or the top or both. All these local inceptions of the disease are in places where callus tissue is actively being formed.

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[1] Smith, Ralph E. The diamond canker disease of the French prune in California. Cal. Agr. Ext. Circ. 67: 22 p. 1932.

In individual orchard trees diamond canker does not, as a rule, become noticeable until they are from 6 to 8 years of age. The earliest cases usually start as in figures 3, A, and 3, D. Canker tissue forms in wound-healing callus on the trunk or at the first forks and, in the worst cases, the condition shown in figure 1, A, develops. After the body becomes thoroughly affected the systemic-appearing progress of the disease upward in the main branches becomes manifest by the appearance of diamond cankers and bark thickening around pruning cuts and cracks in the bark. After the tree is badly affected in this manner numerous very vigorous suckers are sent up from the body and root, and on some of the former, which originate in cankered bark, the symptoms shown in figure 1, D–F, appear. The spread of diamond canker in the tree and the injurious effects of the disease are usually very slow. In all but the worst cases many years may elapse with little visible change; but badly affected trees eventually become stunted and weakened and fall prey to secondary enemies like wood decay, fungi, and insects. Some trees may show cankers only after they are many years old. These are usually at pruning cuts on the limbs and may never develop extensively.

The inception and development of diamond canker in young prune orchards seem, in some instances, to suggest that many of the trees were already affected with the disease in latent condition when planted, or that they were peculiarly susceptible to it. In adjoining blocks of trees of this variety in the same orchard, purchased from different nurseries, the disease sometimes develops abundantly in one block and little or not at all in others. In a number of orchards in a given district, where conditions are apparently alike, some have much diamond canker, others none. Other observations, on the contrary, point to the conclusion that its development in the orchard is governed by local conditions of some sort. In good-size plantings, where diamond canker is present, it often is not distributed with any uniformity in the orchard but tends to develop in certain quite definite areas or foci, with many normal trees between. This is true, even in blocks of trees such as those just mentioned, which seem to be predisposed to the disease. The most obvious peculiarity of these diamond-canker areas is that they are places where conditions are favorable to the occurrence of bacterial gummosis or, as it is popularly termed, ''sour sap.'' Such areas usually are characterized by poor drainage or excessive soil moisture. There appears to be a definite correlation in this respect.

The nature of the causative factor in diamond canker presents a question that cannot yet be said to have been solved. The idea was once common in California that these diamond-canker trees represented a sport or strain of the French prune, rather than a pathological condition, and that all such

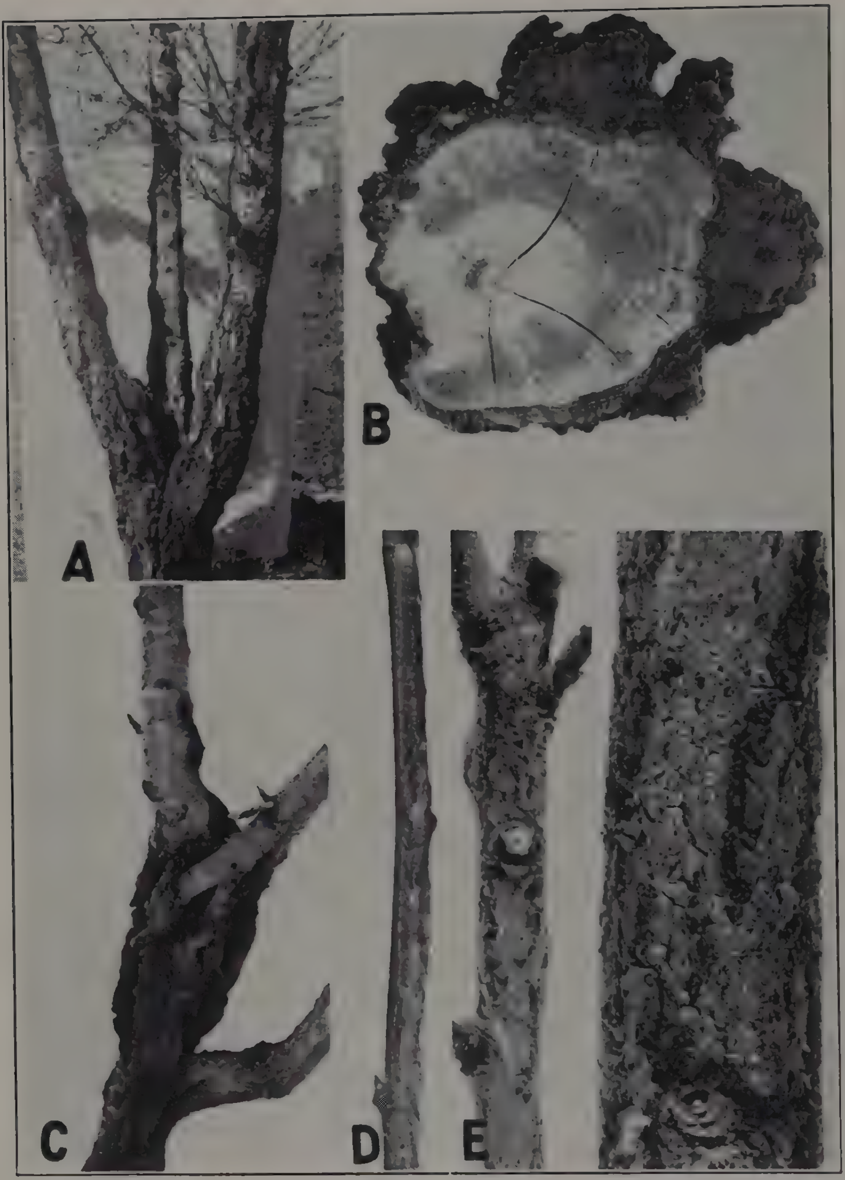

FIG. 1. A. A typical diamond-canker French-prune tree, with rough, swollen bark on trunk and diamond-shape, corky cankers above on main limbs. B. Cross section of a French-prune branch affected with diamond canker, showing that the swelling is composed of corky bark tissue. C. A localized canker on a branch of French prune. Spread of the disease upward is indicated by the appearance of small diamond cankers on the branch above the large swelling. D. Young sprout of French prune, showing symptoms that suggest systemic spread of diamond canker. E. Terminal portion of 3-year-old growth. F. Base of 4-year-old sprout.

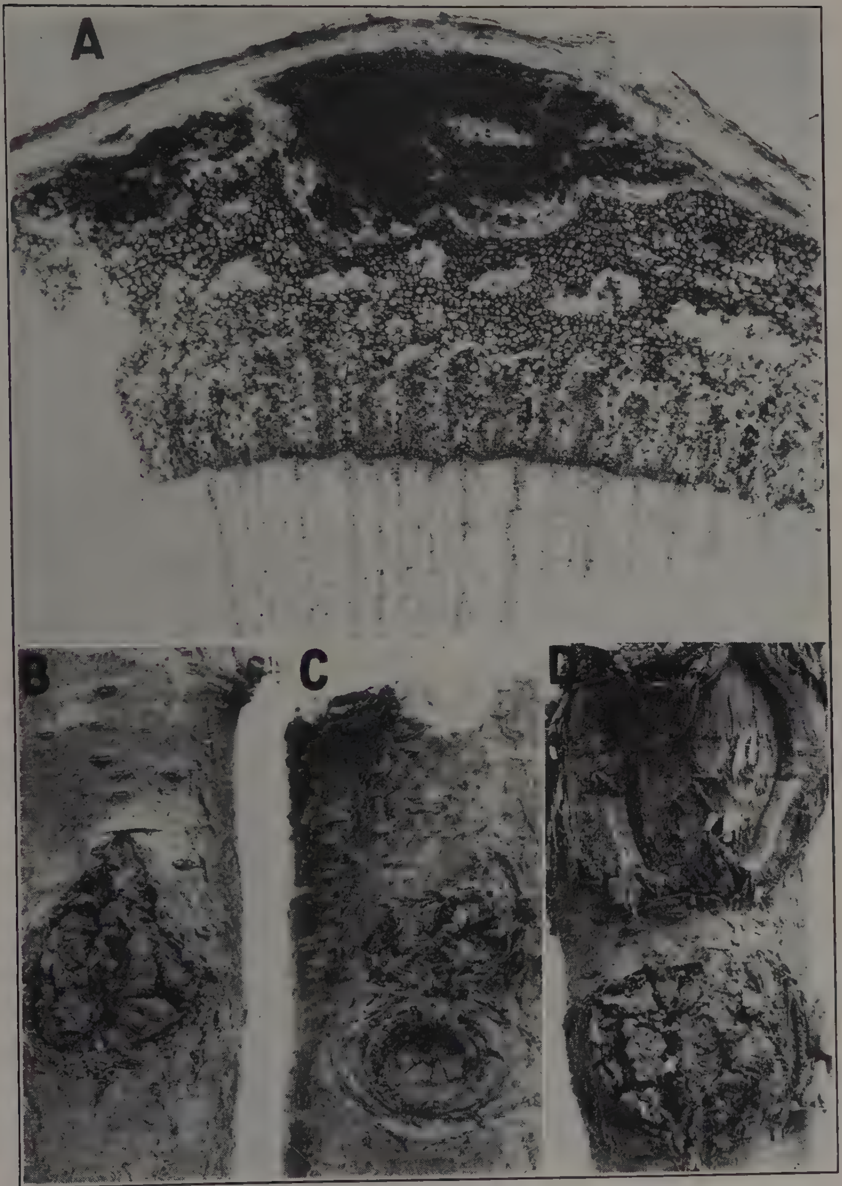

FIG. 2. A. Cross section of 1-year-old French-prune shoot, showing the origin of diamond-canker bark lesions in the phellogen. B. Diamond-canker lesion developing at a pruning cut on limb of French prune. C. Lesion close beside a pruning cut, where the bark apparently was injured when the cut was made. D. Diamond canker originating at a bruise on a limb of French prune. Upper portion of bruise healing normally.

trees, or their parents, had been distributed by one nursery.[2] It is true that some plausibility attached itself to this idea and prune orchards still exist where trees purchased from the nursery in question many years ago have developed much diamond canker when similar trees from other nurseries and planted alongside show none of the disease. It is also true and of possible significance in this connection that the proprietor of this nursery, before and during the period when diamond canker came into prominence, was greatly interested in the search for desirable sports or strains of the French prune and was much addicted to the practise of propagation from individual trees of peculiar characteristics. Inquiry shows, however, that there are in California orchards many diamond-canker prune trees that were propagated and sold by nurseries other than the one mentioned, including a few that antedate it.

Since a large amount and variety of culturing of tissues affected with this condition has been done over a period of years by several different individuals connected with this division, without any results of apparent significance, and since some of the characteristics of the disease, as indicated above, suggest that it may be caused by a virus, grafting and budding experiments were started several years ago in order to determine its mode of transmission.

TRANSMISSION TO SUSCEPTIBLE HOSTS

Two good-size French-prune trees, badly affected with diamond canker, were top-grafted in the main branches about 4 feet above ground, with cions of the following kinds: 1. French prune from a tree where no diamond canker could be seen in this or any other tree in the vicinity; 2. Stewart prune; 3. Sugar prune; 4. Imperial prune; 5. Burton prune. The cut-off limbs of the affected French-prune trees showed very definite bark symptoms of diamond canker up to the point of grafting. All cions grew, and, commencing with the second year, typical symptoms of diamond canker could be seen progressing from the stocks to the growth from the French-prune cions, but not on any of the others. These symptoms became more and more pronounced for 6 years, when the trees were removed. The results of this experiment seem to indicate that diamond canker is a transmissible condition and not a normal, hereditary characteristic of a special strain or sport of the French prune. Also, that the French prune is the only susceptible variety, or at least the only one of those tested, showing visible symptoms of diamond canker within the duration of the experiment.

In regard to the possibility of infecting normal French-prune trees with diamond canker by grafting them with diseased cions, no satisfactory experiments have been completed. Such tests are complicated by the fact mentioned above that diamond canker sometimes develops in French-prune trees top-grafted with strains of the same variety, when both the stock and the tree from which the cions were taken are free from visible symptoms of the disease.

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[2] Baade, H. J. Diamond canker in Napa County. California Cultivator **71**: 494. 1928.

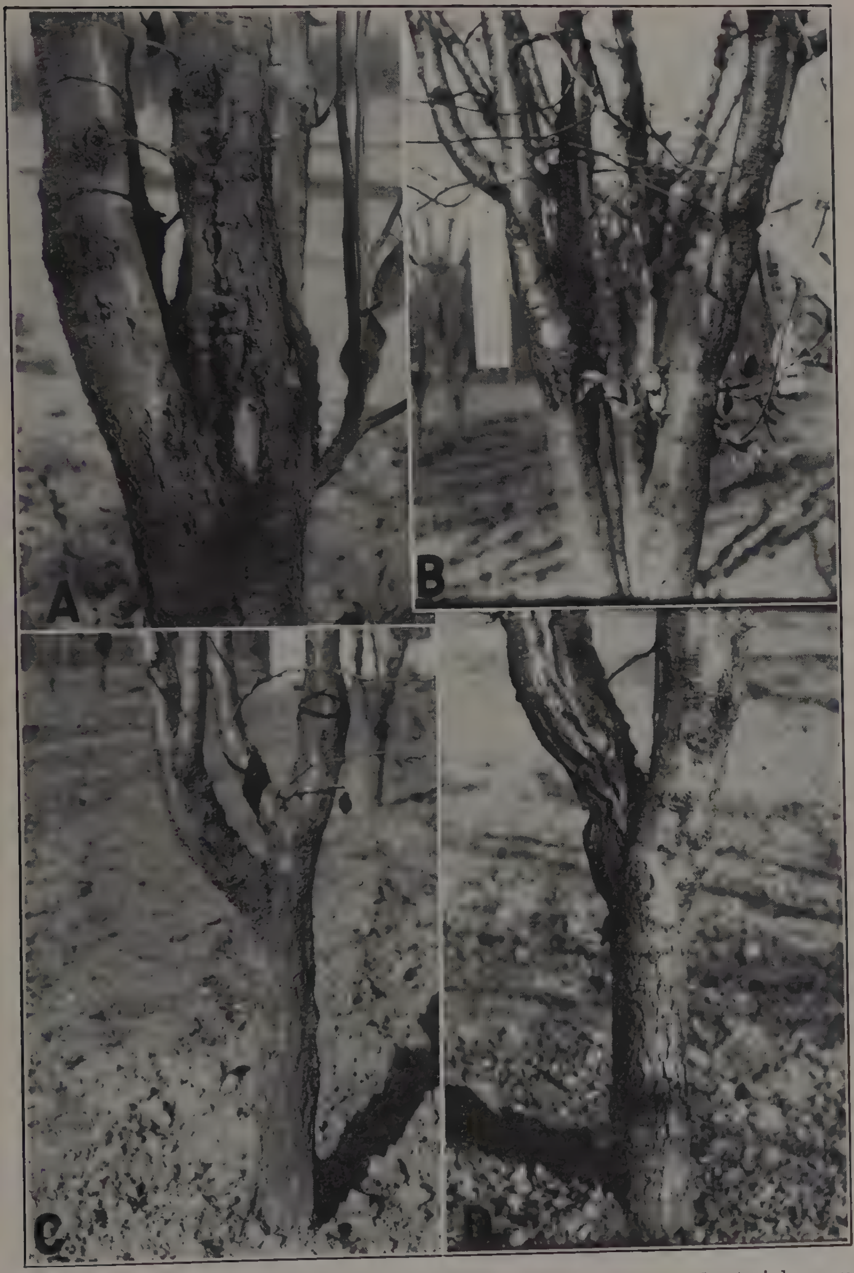

FIG. 3. A. Diamond canker originating in a lesion caused by the bacterial-gummosis organism, *Phytomonas cerasi*. B. Purple myrobalan tree top-grafted in 2 limbs (right) to cions of French prune showing symptoms of diamond canker (no. 1), and (left) 3 limbs with normal cions (no. 2). In this tree, after 5 years, growth from the no. 1 cions showed the disease, but that from the no. 2 cions did not. In 3 trees of 20 thus grafted, diamond canker appeared in both types of cions. C. Seven-year-old French-prune tree, propagated in the nursery from a bud stick that showed lesions of diamond canker. Bark-roughening symptoms of the disease show from the union up to the growth of the current season (Fig. 1, D–F) in every tree propagated from such buds. Trees propagated with buds from normal-looking shoots from the same tree as that from which these diseased shoots were taken, grow normally. D. Diamond canker, which started on a supposedly normal French-prune tree, apparently in a lesion caused by bacterial gummosis.

TRANSMISSION THROUGH NONSUSCEPTIBLE HOSTS

Plantings were made in 1929 of trees of several prunaceous species and varieties like myrobolan, almond, peach, apricot, and several plums, but not including French prune, as material for testing the transmission of the disease through nonsusceptible or symptomless stocks. When these trees were 2 years old cions of French prune were obtained as follows: Lot C, from trees badly affected with diamond canker. Some of the cions showed bark eruptions characteristic of the disease (Fig. 1, D) and others did not. Lots N and S, from apparently perfectly normal trees. Cions of these different lots were grafted on to the various stocks singly and in combination to see if (a) affected cions would produce affected growth, and (b) if diamond canker would be transmitted from a diseased to a healthy cion when they were grafted upon different branches of a nonsusceptible or symptomless stock. One cion of each kind was used per tree.

Symptoms of diamond canker began to show on the growth of some of the C cions after the first year. After 8 years, of 24 trees grafted with these cions the growth from the cions on 16 of them had the disease. This seemed to show no relation to any particular stock.

On 6 trees grafted with N (normal) cions alone, and on 22 trees grafted with both N (normal) and S (normal) the growth was normal.

On 32 trees grafted with S cions alone, the growth from the cions was normal. On 17 trees grafted with both S (normal) and C (diseased) the growth from S on 3 trees and the growth from C on the same trees and most of the others showed diamond canker. This cannot be taken as absolute proof that the pathogenic factor passed through the stock from the affected (C) to the normal (S) cions, as some other observations of the disease do not accord with this conclusion. Further discussion on this point follows later in this paper.

Three years after the first grafting more refined methods were employed by selecting sprouts for cions from 14 different French-prune trees showing various degrees of diamond canker. These included one (no. 1) from a very badly diseased tree with typical bark symptoms on every cion; others, just as badly affected in the trunk and branches with with no visible symptoms on the sprouts; still others exhibiting various degrees of disease but with no sprout symptoms; and one tree (no. 2), about 40 years of age, which appeared to be perfectly free from diamond canker, and in an orchard where none of the trees showed the disease. Some of the myrobolan and other trees were individually grafted with these various cions, and, on a considerable number, cions of both no. 1 and no. 2 were inserted to see if the disease would pass from one to the other through the nonsusceptible stock. Two cions of each kind were used per tree in most cases.

Symptoms of diamond canker began to show on the growth from the no. 1 cions after the first year. After 5 years, of 46 such trees, 39 showed the disease. Twenty-two trees, grafted with no. 2 (Normal) cions alone, and all of the others (nos. 3 to 14), 2 trees with 2 cions of each, showed none of the disease. Of 20 trees grafted with both no. 2 (Normal) and no. 1 (diseased) (Fig. 3, B) 3 showed diamond canker in the growth from the no. 2 cions, as well as in that from almost all of the no. 1. Again it may be remarked that a final conclusion is not justified that the pathogenic factor passed through the stock from the diseased to the healthy cions. If this could occur it seems remarkable that the same pathogenic factor, according to the results of our grafting experiments, is not present in a given shoot more than a very short distance from visible symptoms of the disease. The possibility of the cions having been mixed, or of new infection from the outside having occurred cannot be absolutely excluded. It may be noted, however, that out of almost 250 supposedly normal cions used in all these experiments, the total number (6) that developed diamond canker were all on trees[3] that had also been grafted with diseased cions.

In another experiment, nursery-budded French-prune trees were propagated by the following procedure: (C) Bud sticks from sprouts taken from a tree badly affected with diamond canker and with bark symptoms present on every stick (Fig. 1, D). (D) Bud sticks from a similarly affected tree, but with no visible symptoms on the sprouts. (B) Sticks from an apparently normal tree in the same orchard. These buds were turned over to a nursery with instructions to propagate about 50 trees of each lot on their regular myrobolan seedling roots. When the trees were ready for delivery 18 months later another lot of 50 trees (A) of the nursery's regular commercial French-prune trees of the same age was purchased. These 4 lots of prune trees, 225 trees in all, were planted out in orchard form in February, 1934.

The second year later, trees in lot C began to show diamond canker in the trunk above the point of budding, and eventually every tree in that lot developed the disease very badly in this manner (Fig. 3, C). By 1941 these 50 trees were all very badly stunted or dead. All the trees in the other lots grew normally and showed no trace of diamond canker up to 1941, except 1 tree in lot B. In this there began to appear in 1938 a large, typical canker at the base of one of the main limbs about 2 feet above ground (Fig. 3, D). This canker started in a bacterial gummosis lesion, and had all the appearance of a local wound infection. Symptoms of the disease ran up this limb in the typical, systematic-appearing manner and its growth was noticeably stunted. The trunk and the other limbs showed no signs of diamond canker up to 1941. All the remaining trees in all lots, except C, had made a normal growth up to this time.

Another set of trees, similar to the last, was propagated and set out in orchard form in 1936. In this experiment lot G corresponded to lot D (normal-looking bud sticks from a badly diseased tree); lot H was the same as lot B (bud sticks from a healthy-looking tree in a badly affected orchard); and lot I was the same as lot C (bud sticks showing diamond canker symptoms). The second year some of the trees in lot I began to show the disease in the trunk, just above the union; by the next year they were all badly affected. None of the other trees had shown any diamond canker up to 1941.

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[3] These were various myrobolans, Marianna, and St. Julian G.

DISCUSSION

There are at least 3 initial possibilities concerning the nature of diamond canker: 1. That it is a hereditary sport or strain of the French prune; 2. That it results from external infection with some pathogenic factor; 3. That it is a virosis. Of these possibilities numbers 2 and 3 are not necessarily opposed to each other. That this condition is not a sport seems to have been proved by the first experiment described, where the growth from normal French-prune cions contracted diamond canker when grafted on affected trees. It is significant also that cions or buds taken from diamond-canker trees did not produce this type of growth (at least not within 8 years) unless visible symptoms were present on the parts used for propagation. Further evidence that the diamond-canker condition results from some external factor is furnished by the fact that sometimes, in grafted French prunes, even with stocks or cions of the normal type, the disease develops.

Much doubt is cast upon the possibility of the causative factor being a pathogenic microorganism like a fungus or bacterium, by the non-success of much culturing, and by the apparently systemic, virus-like spread of the diamond-canker condition in badly affected trees. It is very difficult to conceive that any known type of microorganism could develop in the growth of young prune shoots in the manner illustrated in figures 1, D–F, and 2, A, to a distance of as much as 6 feet in a single growing season. Moreover, the development of the disease in a young tree (Fig. 3, C) or a graft (Fig. 3, B) propagated from lesion-bearing material is decidedly of a systemic type and not like the spread of a microorganism. If, however, we are dealing with a virus, as most of the facts indicate, the fact that buds from symptomless shoots taken from trees badly affected with diamond canker do not produce diseased growth, at least not within several years, indicates that such a virus must be localized in symptom-bearing tissue or quantitatively restricted to an unusual extent.

The original inception of the disease in individual trees cannot, at present, be fully explained. While cases like those shown in figures 3, B, and 3, C, can be produced in nearly every instance by budding or grafting from symptom-bearing shoots, it is incredible that commercial nurseries would use such bud sticks to any extent, since they not only look diseased but also are hard to find in any quantity. Furthermore, in trees grown from such buds symptoms of diamond canker appear promptly at the union and extend quite uniformly upward in a systemic manner, whereas cases of the disease in orchard trees start most commonly as shown in figures 2, B–D, and 3, A and D, and appear like local outgrowths from wound callus. Why this should occur abundantly in some and not at all in other, even adjacent, prune orchards is another unanswered question. In this and other respects diamond canker shows much resemblance to the disease called psorosis or scaly bark of citrus trees, which, in the opinion of Fawcett,[4] Rhoads,[5] and Doidge,[6] is transmitted exclusively by budding from or upon affected trees, but which may remain latent in the tree for as much as 30 years (Doidge) before showing bark symptoms. Even though the causative factor is present in the tree, soil and growth conditions may greatly influence the visible manifestation of symptoms (Fawcett).

SUMMARY

A disease of French-prune trees in California is described. No other species or variety of tree is known to be susceptible.

The trouble is characterized by rough, corky thickening of the bark on the trunk and branches, with diamond-shape excrescences at places like pruning cuts or bark cracks.

These symptoms usually are first seen on a tree in places where wound callus is forming.

In badly affected trees the disease has the appearance of becoming systemic.

Many attempts to isolate a causative organism from diseased tissues by culturing methods have failed.

The idea that diamond-canker trees represent a hereditary sport or strain of the French prune has been disproved, since the disease is transmitted to normal grafts growing upon cankered stocks.

When buds or cions were taken from affected trees the disease appeared promptly in the resulting growth, if bark lesions were present in the parts used for propagation.

When normal-looking buds or cions taken from the same or other diseased trees were used, the resulting growth always appeared normal for the duration of the experiments (8 years).

When normal and cankered cions were grafted on opposite sides of the same nonsusceptible stock, the disease in a few cases appeared in the growth from the normal grafts.

Diamond canker of the French prune apparently is caused by a localized virus and shows some resemblance in its behavior to the disease of citrus trees called psorosis or scaly bark, which has been reported in California, Florida, and South Africa.

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[4] Fawcett, H. S. Scaly bark in relation to propagation of citrus trees. Calif. Citrograph 24: 242–262. 1939.

[5] Rhoads, A. S. Further light on the nature and cause of psorosis of citrus trees. Citrus Indus. 20: 11, 12, 14. 1939.

[6] Doidge, Ethel M., and F. A. S. Turner. Psorosis or scaly bark of citrus trees. Farming in So. Africa 14: 363–364. 1939.

CYTOSPORA CANKER OF ITALIAN CYPRESS[1]

GEORGE A. ZENTMYER[2]

(Accepted for publication January 19, 1941)

During the past 12 years a canker disease of the columnar Italian cypress (*Cupressus sempervirens* L. var. *stricta* Ait.) has become increasingly prevalent along the southern and central California coast and has assumed serious proportions in certain regions, taking a heavy toll of this ornamental cypress. This paper presents the results of an investigation on the disease begun in 1935, at the University of California, during the course of which the pathogenicity of a species of *Cytospora* commonly associated with the lesions was definitely established.

THE DISEASE

History and Distribution

The canker was first observed in 1928 in Berkeley and San Diego, California, two coastal cities about 450 miles apart. At this time diseased material was sent in by property owners to the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, at San Francisco. The fungus associated with the lesions was proved to be a species of *Cytospora* by W. W. Wagener, who made subsequent observations on the disease (13, 14).

The disease appears to be limited in occurrence to a narrow belt along the California coast; the distance inland to which it occurs varies with topography but usually has not been found to exceed 4 or 5 miles. It occurs at several widely separated localities along the coast: San Diego, Oceanside, the Los Angeles beach area, Carpenteria, Santa Barbara, near San Luis Obispo, and in the Monterey and San Francisco Bay regions (Fig. 1).

Hosts Naturally Affected

The columnar form[3] of *Cupressus sempervirens* is the principal host of the parasite. The *Cytospora* species involved has also been isolated from the horizontal form (*C. sempervirens* L. var. *horizontalis* Gord.), from Monterey cypress (*C. macrocarpa* Hartw.), and from smooth cypress[4] (*C. glabra* Sudw.), but it is actively parasitic on only the first of these last three hosts. The horizontal form of *C. sempervirens* is not commonly grown in

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[1] Revision of thesis submitted to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree Doctor of Philosophy. The writer wishes to express his deep appreciation to Dr. H. N. Hansen of the Division of Plant Pathology, University of California, Berkeley, Calif., for his supervision of the work, and to Dr. W. W. Wagener of the Division of Forest Pathology, U. S. Department of Agriculture, San Francisco, Calif., for suggesting the problem and for valuable criticism.

[2] Part of the work was done while the writer was Assistant Pathologist, Division of Forest Pathology, U. S. Department of Agriculture, San Francisco, Calif.

[3] This form is commonly referred to simply as "Italian cypress." Where this term is used in this paper the columnar form is to be understood.

[4] The name "Arizona cypress" is commonly used in California.

California; hence its susceptibility is of little significance in the region. *C. glabra* is apparently relatively resistant to attack by the fungus or to development of the pathogen, even after invasion of the cortical region; the few cankers found on this host in California had caused little damage. The fungus has been found occasionally on planted Monterey cypress as a saprophyte or weak parasite, associated with dying back of branch tips, and gives no indication of being important in the pathology of this tree. In many cases the fungus becomes established on Monterey cypress following injury to branch tips by fire, salt spray, or wind; after thus gaining entry to the host it forms limited lesions that extend slowly down the branches.

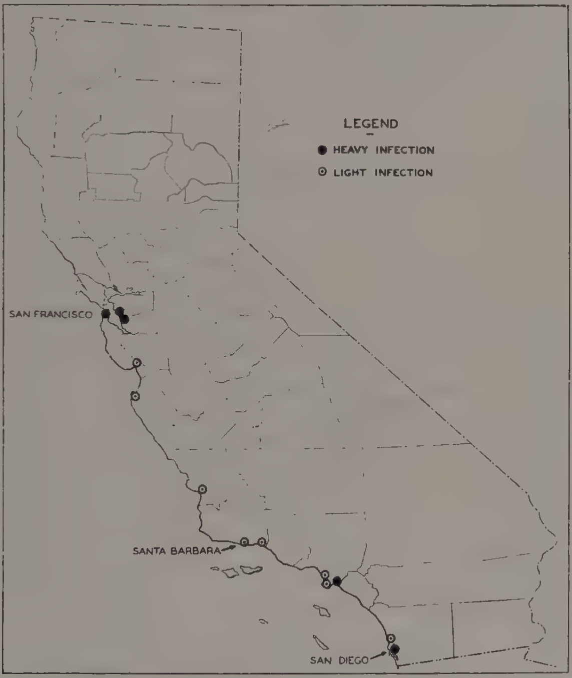

FIG. 1. Known distribution of Cytospora canker on Italian cypress in California in 1940. Italian cypress is widely planted in California, in the inland regions as well as along the coast.

All of these cypresses are used as ornamental in California arboriculture. Three of them are exotics. Monterey cypress is indigenous to the State, growing naturally in very localized groves in the Monterey Bay region. The horizontal Italian cypress is native to the general region of southeastern Europe and southwestern Asia (the Mediterranean region) (1, 5), whereas the columnar form appears to have originated from this horizontal type in cultivation. *Cupressus glabra* occurs in the natural state in the southwestern United States.

Description

The first gross symptom of the disease is the appearance of yellow-green foliage on branches bearing the girdling cankers. The foliage turns more

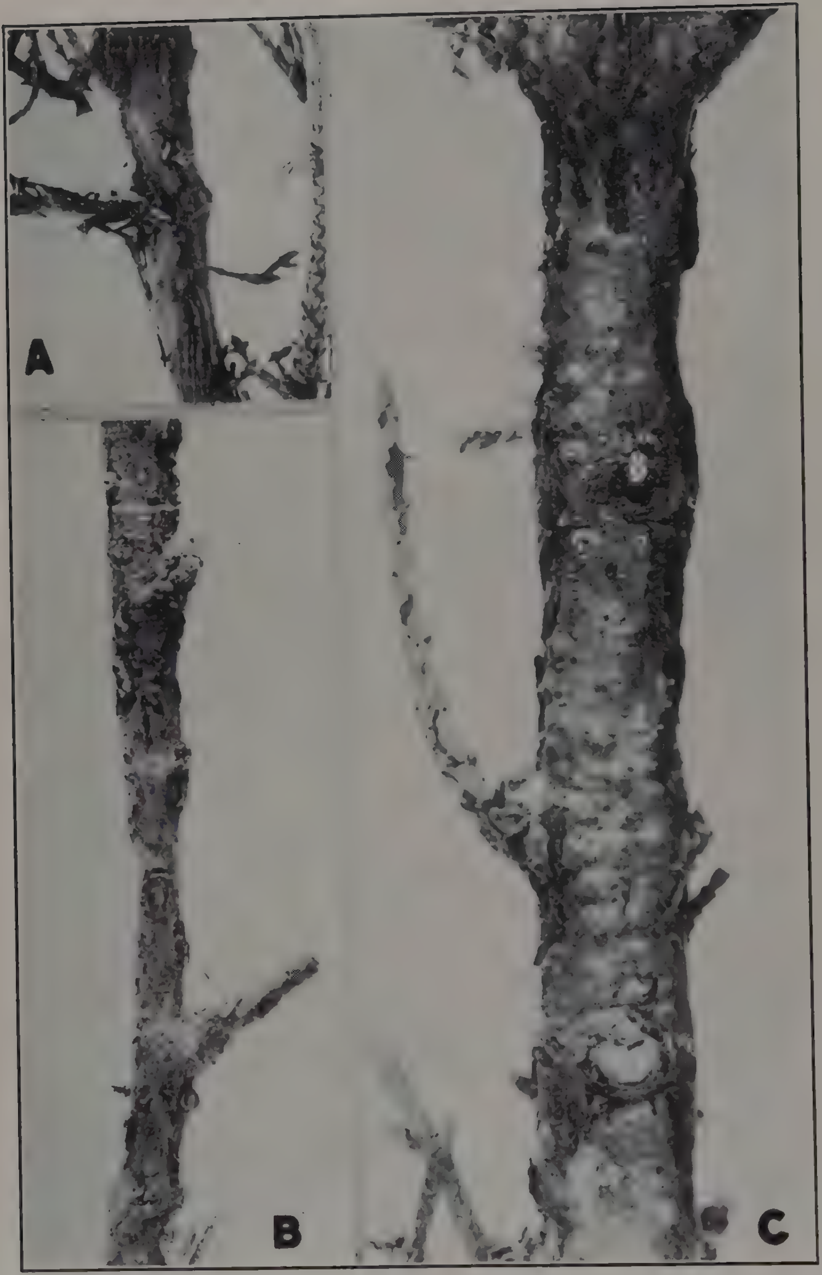

FIG. 2. Natural cankers caused by *Cytospora cenisia littoralis* on Italian cypress. A. Young canker centering around small dead lateral. ×1. B. More advanced canker showing constriction, cracking of bark, resinosis, and stromata. ×1. C. Enlarged portion of canker showing stromata. ×3.

yellow and finally becomes brown and persists on the branches for some time after death, so that these brown "flags" stand out in contrast to the normal green foliage. Young branch cankers are smooth and reddish-brown, often accompanied by slight constriction of the branch and by slight, abnormal resin flow (Fig. 2A). Stromata of the pathogen on young cankers are barely visible to the naked eye, appearing as minute greenish-topped pustules on the reddish-brown cankered surface. The fungus apparently is strictly caulicolous; it has never been found on cypress foliage. In many cases small dead branches have been found at the center of young cankers; the facultative parasitism exhibited by the fungus in connection with its growth on Monterey cypress indicates that it may enter the trees through such branches.

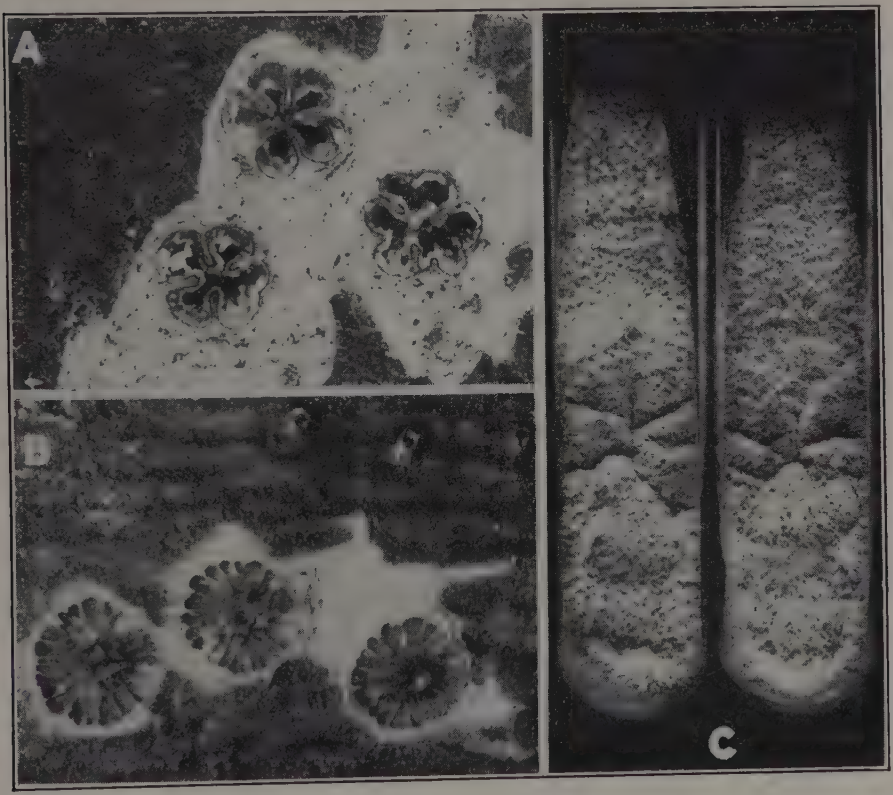

FIG. 3. A and B. Comparison of cross sections of stromata of *Cytospora cenisia littoralis* (A) and of a saprophytic species of *Cytospora* (B) growing on Italian cypress. Note the unsectioned stromata in the upper right corner of B. ×15. C. Mono-conidial cultures of *C. cenisia littoralis* one month old, on potato-dextrose agar. ×1.

On older branches the bark becomes more cracked and distorted, with increased resin flow resulting (Fig. 2, B). Resin infiltration in the bark is not nearly so heavy as in the case of Coryneum canker of Monterey and Italian cypresses, caused by *Coryneum cardinale* Wagener (15). The stromata, formed just beneath the phellogen, enlarge and many new fruiting bodies are formed until old cankers become thickly dotted with them (Fig. 2, C). When typical, mature stromata of the fungus are sectioned horizontally the rather simple locular structure (Fig. 3, A) and the characteristic strontian yellow (8) of the enclosed spore mass are readily evident. Cirrhi do not constitute a common diagnostic feature of Cytospora cankers on Italian cypress, though, occasionally, during humid weather, globular spore masses are seen at the ostiole.

Damage

As an example of possible severity of attack, as many as 30 branch cankers, in addition to several large trunk cankers, have been observed on a 25-ft. Italian cypress. Trees 30 ft. high and 6 in. in diameter at the base have succumbed within a year after entrance of the fungus by natural means into the main trunk. Trees up to 10 ft. high have died within 11 months after inoculation.

Infection does not progress so rapidly as this in all cases, however, thus indicating the presence of different strains of the fungus, or of variations in host susceptibility, which may be hereditary or the result of some physiological difference. The canker is usually more destructive on trees weakened in any respect; in this connection, Italian cypress grows poorly where immediately exposed to ocean winds and salt spray; but this type of exposure is not common to all localities where the canker occurs. Young trees up to 5 years old have never been found diseased, possibly because of a combination of rapid, vigorous growth in such trees and practical absence of dead branches that, had they been present, might have served as points of entry for the fungus.

A small area less than ½ mile square in north Berkeley, California, on which disease conditions were closely watched from 1936 to 1938, furnishes an example of the prevalence and virulence of the fungus in this typical coastal locality. In 1936, 32 of the 62 Italian cypresses in the area were diseased. By 1938 20 trees had been removed because of Cytospora canker, and 29 of the remaining 42 cypresses were diseased. The columnar Italian cypress is losing favor as an ornamental tree in coastal cities because of the high mortality and the unsightliness of diseased trees. It is rather difficult to find an adequate substitute in the columnar ornamental tree field; very few other trees are adaptable for use in the formal type of planting, so that elimination of Italian cypress in coastal cities by Cytospora canker would be a distinct loss.

ETIOLOGY

Inoculation Experiments

Isolations have been made from over 50 diseased cypresses from various localities along the California coast; the same species of *Cytospora* has consistently appeared in culture. No appreciable variation in cultural characteristics has been observed, either in mass or monoconidial isolates.

Sixteen 5-year-old Italian cypresses were inoculated by the slit method described by Meinecke (6) at 2-week intervals during the fall of 1936 and the spring and early summer of 1937. Several inoculations and one check slit were made on each tree. In most cases mycelium and a small piece of attached agar from the culture were introduced into the slit in the cortical tissue; in a few instances highly concentrated suspensions of conidia were placed in the cuts. The inoculation cuts were wrapped with adhesive tape to prevent too rapid desiccation.

Of 50 inoculations made at different times during the year, 38 resulted in production of cankers with consequent resin flow and formation of stromata; cankers were formed on 14 of the 16 trees inoculated. Two of the trees died within 12 months, and canker development was sufficient on 9 other trees at the termination of the examinations to enable one to predict their early death. Reisolation confirmed the pathogenicity of the species of *Cytospora,* as the same organism was recovered in culture. The checks were all negative. Of the 39 mycelial inoculations made between September 16, 1936, and April 15, 1937, 34, or 87 per cent, were successful. Of the 11 mycelial inoculations made between April 29 and July 25, 1937, only 4, or 36 per cent, were successful; in addition, 8 inoculations, using spore suspensions during this latter period were unsuccessful. The method of protecting the cuts after inoculation, merely covering them with adhesive tape, was not intended to provide highly favorable unnatural conditions to aid the fungus in its development, and with the advent of warmer weather the inoculum may have dried too rapidly.

Two mycelial inoculations were made on each of 3 small Monterey cypresses. In each case a small, definitely limited canker was formed; these cankers extended but a short distance in 18 months and the fungus could not be recovered from them, indicating that the host had successfully walled off the incipient infections.

The Causal Organism

Morphological and cultural comparison has been made between the pathogen and the species of *Cytospora* described on members of the family Cupressaceae and other available species on various other coniferous hosts.[5] Four species of *Cytospora* have been described from *Cupressus* sp.: *Cytospora cupressi* Gutner (4), *C. cupressina* Sousa da Camara (12), *C. pinastri* Fr. (2, 3), and *C. pini* Desm. (3); each is very distinct from the Italian cypress pathogen, mainly because of the occurrence of numerous locules in the stromata, although other differences are apparent.

The species of *Cytospora* with which this paper is concerned closely resembles specimens of *C. cenisia* Sacc. and *C. dubyi* Sacc., which have been described as saprophytes on *Juniperus* sp. in North America and Europe, respectively (7, 9, 10). The main features of the short descriptions given

[5] The writer is indebted to Dr. A. M. Waterman, Division of Forest Pathology, U. S. Department of Agriculture, New Haven, Conn., for furnishing a number of the cultures; and to Professor John Dearness, London, Ontario, for suggestions and information on conifer-inhabiting *Cytospora* species.

for *C. cenisia* by Nitschke (7) and Saccardo (9) coincide closely with those of the Italian cypress pathogen. Exsiccati specimens of *C. cenisia* from *Juniperus virginiana* and specimens of this fungus collected from the same host in Connecticut resemble the California *Cytospora* morphologically, with minor variations, but are culturally different. The minor morphological variations include the presence of a very definite border of tissue delimiting the outer edge of the stroma in the case of the Italian cypress pathogen (Fig. 3, A), whereas in *C. cenisia* the periphery of the stromatal tissue is not so definitely outlined; and the extension of the conidiophores of the cypress pathogen is farther into the locular cavity than is the case with *C. cenisia.*

The descriptions of *Cytospora dubyi* (7, 9) include stromata with many small locules as one of the primary characters; all of the exsiccati specimens examined bore stromata with few locules resembling those of *C. cenisia* and of the Italian cypress pathogen. Because of this discrepancy and the close morphological resemblance between the Italian cypress pathogen and *C. cenisia,* it was considered advisable to designate the pathogen as a form of *C. cenisia.*

Cytospora cenisia Sacc. forma *littoralis* forma nov.—Stromata scattered on young cankers, becoming gregarious and even closely crowded on older lesions, innate and finally erumpent with the greenish-gray truncate ostiole protruding through the epidermis, developing in cortical tissue beneath the phellogen, 0.50 to 1.00 mm. in diameter and 0.25 to 0.50 mm. in thickness; locules few (4–7), large (300 to 600 microns diameter), bounded by a definite margin of dark stromatal tissue, usually radiating, with separate necks leading into a small common ostiole; conidiophores in clumps, often extending some distance into the cavities, simple or branched, 8.5–15 × 1.5–2 microns; conidia hyaline, allantoid, 4.6(3.0–7.2) × 1.0(0.6–1.5) microns, conidial masses usually strontian yellow.

Habitat—caulicolous parasite on *Cupressus* spp. along the coast of southern and central California; forming cankers on branches and trunks of *C. sempervirens* L. var. *stricta* Ait., of less importance on *C. sempervirens* L. var. *horizontalis* Gord., *C. macrocarpa* Hartw., and *C. glabra* Sudw.

Stromatibus erumpentibus, 0.5–1 mm. in diam., 0.25–0.5 mm. crassis; loculis magnis, 300–600 μ diam., paucis (4–7), radiantibus, linea atra definita circumdatis; conidiophoris caespitosis, simplicibus vel ramosis, 8.5–15 × 1.5–2 μ; conidiis allantoideis, hyalinis, 4.6 (3.0–7.2) × 1.0(0.6–1.5) μ; massa conidica citrina.

In ramis et truncis *Cupressi sempervirentis* var. *strictae* et var. *horizontalis, C. macrocarpae,* et *C. glabrae,* cancros formans; California.[6]

Specimens have been deposited in the herbarium of the Division of Plant Pathology, University of California, Berkeley, Calif.; and in the mycological collections of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. Specimens are also filed in the collections of the Division of Forest Pathology, San Francisco, Calif.

C. cenisia littoralis has a rather simple type of loculation with usually only 4 to 5 large main locules (pycnidia) evident on sectioning the mature stroma, and is further characterized by a prominent border of stromatic tissue bounding the fruiting body (Fig. 3, A). This arrangement is in striking contrast to the locular picture in most of the saprophytic species of *Cytospora* of which specimens were examined; these usually have large numbers of small radiating locules around a central column (Fig. 3, B).

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[6] The writer is indebted to Edith Cash, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture for the Latin description.

The conidiophores of the pathogen occur in clumps. Groups of them often extend some distance out into the pycnidial cavity, giving the appearance of poorly developed loculation within the pycnidium itself.

Spore germination is of the type apparently common to all species of *Cytospora*. Germination is satisfactory on agar plates; the spores swell to from 1½ to 2 times their length and from 3 to 5 times their width within 48 hours, and become nearly ovoid, losing all resemblance to the original allantoid form. Germ-tube formation is commonly monopolar, but occasionally it is bipolar; branching is not common on tubes less than 90 μ long.

The structure of the stroma has been found to be a reasonably constant differentiating character in the species of *Cytospora* studied in this investigation. Diedecke (2) and Gutner (4) have maintained that classification of members of the genus *Cytospora* should be based on the structure of the stroma, *i.e.*, the number and arrangement of locules within the fruiting body. This character is of taxonomic value chiefly if horizontal sections of the stromata are studied, as use of vertical sections alone gives too much opportunity for variation of the locular picture with varying orientation of the stromata.

It would seem that the confused taxonomy of the genus *Cytospora* should be clarified by revision of the genus along the lines proposed for the genus *Fusarium* by Snyder and Hansen (11). In the case of the genus *Cytospora* the structure of the stroma would be one of the primary morphological characters in formulating species, while physiological differences would serve as bases for designating forms, following the method used by Snyder and Hansen in reclassifying the section Elegans of the genus *Fusarium*. Such a method would reduce the present inordinately large number of species of *Cytospora* to a small group that could be worked with convenience. It is with this idea in mind that the Italian cypress pathogen is given a form name in the species *Cytospora cenisia*.

Temperature Relations

The Italian cypress pathogen is a rather low-temperature organism, as shown by measurement of growth of several isolates in temperature chambers set at 3-degree intervals. These measurements were supplemented by further temperature studies at the upper end of the range, using chambers set at 1-degree intervals. Conclusions were based on the average growth of 3 plates for each of 3 isolates at each temperature. The minimum temperature for growth of *C. cenisia littoralis* was found to be approximately 1° C., the optimum close to 19° C., and the maximum 25° C. Average colony diameters after 13 days growth on agar plates were as follows: 1°, 0 mm.; 4°, 10 mm.; 7°, 19 mm.; 10°, 37 mm.; 13°, 55 mm.; 16°, 68 mm.; 19°, 90 mm.; 22°, 83 mm.; 25°, 6 mm.; 28°, 0 mm. In contrast to the pathogen, 2 saprophytic species of *Cytospora* from Italian cypress and several other species of *Cytospora* of more cosmopolitan distribution grow well up to 34° C.

This low temperature requirement of the pathogen seems related to the known occurrence of the disease only in the cool littoral zone in California. Study of temperature records at various localities along the California coast, as compared with inland stations, lends factual support to the above indication. For several stations the average number of hours per year with temperature of 25° C. or over and of 1° C. or under was determined, based on thermograph records for the period 1934–1936, inclusive. In two coastal cities, San Diego and San Francisco, where the disease is common and destructive, it was found that during only 1.5 and 1.2 per cent of the hours per year, respectively, was the temperature unfavorable for growth of the fungus. The inland cities, on the contrary, present temperature conditions increasingly less favorable to the fungus as the distance from the coastal region increases (Table 1). The climate of the coastal region is influenced by the prevailing westerly winds blowing from off the ocean; hence it is of the maritime type. At varying but relatively short distances inland this influence is disturbed by the presence of foothills and by the main Coast Range Mountains, with the result that the inland regions are characterized by considerably greater variations in temperature, little fog, much warmer summer temperatures, and higher average temperatures.

TABLE 1.—*Temperature in relation to presence of Cytospora canker at several localities in California*

Station	Distance from ocean (miles)	Disease conditions	Average hours per year with temperature over maximum or under minimum tolerated by *C. cenisia littoralis*	
			No.	Per cent
San Diego	$\frac{1}{4}$[a]	Canker present	133	1.5
San Francisco	$\frac{1}{2}$[a]	Canker present	104	1.2
Los Angeles	13	No canker found	737	8.4
San Bernardino	50	No canker found	1848	21.0

[a] Distance from bay. Distances based on locations of U. S. Weather Bureau stations during the period 1934–1936.

Cultural Character

Cytospora cenisia littoralis grows readily on a wide variety of culture media, with best growth on potato-dextrose and malt-extract agars. Stromata develop on several media; most rapid and abundant formation occurs on sterilized elderberry or fig stems, on sterilized oats and wheat, and on malt-extract agar. The mycelium grows slowly, first appearing rather sparse. Upon full development on potato-dextrose agar the mycelium is closely compacted, irregularly zonate, with some aerial hyphae growing in tufted fashion, white with occasional streaks of pinard yellow (8), and with small greenish stromata formed along the edges and in the lower third of the slant (Fig. 3, C). The agar becomes convoluted at the point of inoculation but undergoes no color change. Cirrhi are not commonly formed in culture on the various agar media; they occasionally exude from the fruiting bodies after two or three months growth on potato-dextrose agar. They are numerous on stromata formed on steamed oats or wheat.

Canker Growth Increment

Measurements of canker growth were conducted on inoculated and on naturally infected trees in Berkeley. The average increase in length attained by 12 of the natural-infection cankers in 3 months was 14.7 cm., with the range of increase from 4.5 to 32.0 cm. These measurements involve cankers on various-size stems, but they all cover the period from July 18 to October 17, 1937. The average length of 38 artificially produced cankers in 3 months was 5.2 cm., with lengths ranging from 1.5 to 14.0 cm. at the end of that period. These measurements were made on cankers resulting from inoculations on stems of different size and at different times of year. Inoculation slits on these trees were usually from $\frac{1}{2}$ to $\frac{3}{4}$ cm. long.

CONTROL MEASURES

Although no work has been done on control measures, certain suggestions can be made on the basis of observations during the course of this investigation. Thorough spraying of a dense-foliaged tree, such as Italian cypress, is rather difficult, hence other preventive measures should be used to keep trees free from canker. Wounding should be avoided, and pruning cuts should be protected with some substance such as Bordeaux paint with linseed oil, or asphalt paint. Chafing injuries from supporting wires or ropes and injuries at the base of the trunk made by garden tools should be guarded against in particular. Branches that are shaded out or that die from other causes should be removed at the point of connection with living tissue to prevent the fungus from entering through them. In established cases of disease, side branches bearing cankers should be cut off at least 6 inches below the last sign of the canker, and the wounds protected as mentioned above. In the case of well-developed cankers on the main trunk the safest policy is to remove the entire tree and burn it.

Spread of the disease over wide distances in California has apparently been due to entry of diseased nursery stock into previously disease-free areas. If Italian cypress cultivation is continued to any extent, close watch should be kept of disease-free districts, and immediate eradication of any cases of disease should do much to control the canker. Spread is not so rapid nor are all specimens so severely attacked but that district sanitation measures could provide control. Wind apparently plays little if any part in distribution of the organism, on the basis of negative results in the exposure of agar plates near infected trees following periods of rainfall. Local spread is apparently largely traceable to dissemination of spores on pruning tools, to washing and splashing of spores by rain, fog, and dew, and to some extent by a natural agent, the identity of which has not been established.

SUMMARY

A canker disease of the columnar variety of *Cupressus sempervirens* (*C. sempervirens* L. var. *stricta* Ait.) caused by *Cytospora cenisia* Sacc. forma *littoralis* forma nov. is described. Cankerous lesions, with accompanying resinosis, are formed on branches and trunks of this ornamental cypress; the disease occurs in the coastal regions of southern and central California and has not been found more than four miles from the coast line in these regions. The disease has been present for at least 12 years in the San Diego and San Francisco bay regions.

C. sempervirens stricta, the Italian cypress, is the principal host of the pathogen. The disease also occurs on the horizontal form of *C. sempervirens*, on *C. glabra* to a limited extent, and the fungus has been found on Monterey cypress as a saprophyte or weak parasite.

In certain localities the disease has assumed epiphytotic proportions. Many trees have been killed by the pathogen and others have been ruined for ornamental purposes. Inoculations on 16 Italian cypresses ranging from 5 to 10 feet in height resulted in the death of 2 of the trees within 12 months and in severe canker development on 9 other trees.

The pathogen will not grow in culture at temperatures above 25° C.; the optimum is near 19° C. This fact apparently has a bearing on the occurrence of the disease only in the cooler coastal regions of California.

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LITERATURE CITED

1. CAMUS, A. Les Cypres. 106 pp. Paul Lechevalier, (Paris). 1914.
2. DIEDECKE, H. Pilze VII—Sphaeropsideae, Melanconieae, 962 pp. *In:* Kryptogamenflora der Mark Brandenburg, (Leipzig) 9: 1915.
3. GROVE, W. B. I. The British species of *Cytospora*. Kew Misc. Inform. Bull., 1: 1–30. 1923.
4. GUTNER, L. S. Contributions to a monograph of the genus *Cytospora*. Acta. Inst. Bot. Acad. Sci. 2: 411–484. 1935. (Russian with German summary).
5. JOUBERT, H., and P. A. BUROLLET. Biologie et rôle forestier des Cypres. Rev. Eaux et Forets. 72: 85–93. 1934.
6. MEINECKE, E. P. Facultative heteroecism in *Peridermium cerebrum* and *Peridermium harknessii*. Phytopath. 10: 279–297. 1920.
7. NITSCHKE, T. Pyrenomycetes Germanica (Breslau). 1: 320. 1867.
8. RIDGWAY, R. Color standards and color nomenclature (Washington). 43 pp., 53 colored plates. 1912.
9. SACCARDO, P. A. Sylloge Fungorum. 3: 1–458. 1884.
10. ————. Notae mycologicae. Ann. Mycologici. 13: 114–138. 1915.
11. SNYDER, W. C., and H. N. HANSEN. The species concept in *Fusarium*. Amer. Jour. Bot. 27: 64–67. 1940.
12. SOUSA DA CAMARA, E. DE. Mycoflora Lusitanica. Centuria X., Rev. Agron. (Lisboa) 20: 1–32. 1932.
13. WAGENER, W. W. An outline of tree disease problems in California. Proc. Western Shade Tree Conf. 1: 14–21. 1934.
14. ————. The cypress bark canker and other cypress diseases. Proc. Western Shade Tree Conf. 3: 79–85. 1936.
15. ————. The canker of Cupressus induced by **Coryneum cardinale**, n. sp. Jour. Agr. Res. [U. S.] 58: 1–46. 1939.

HOST–PARASITE RELATIONSHIPS IN PINK ROOT OF ALLIUM CEPA. II. THE ACTION OF PHOMA TERRESTRIS ON ALLIUM CEPA AND OTHER HOSTS[1, 2]

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INTRODUCTION

Pink root is one of the serious diseases of the onion in Colorado. Its causal agent has been found in the soils of a majority of onion fields on the Eastern Slope of the State, and in a few on the Western Slope.

Although this disease has received the attention of certain investigators (1, 2, 3), a review of the research thus far conducted emphasizes the need for more knowledge on the relation of the causal agent, *Phoma terrestris* Hansen, to the onion and to other possible host plants. In addition more information is needed on the rôle of *P. terrestris* as a possible agent predisposing onion bulbs to attack by *Fusarium vasinfectum* var. *zonatum*. It is the purpose of this paper to present certain findings relative to these subjects.

EXPERIMENTAL

The Action of the Pathogen on Onion Roots

In order to investigate the action of *Phoma terrestris* on onion-root tissues, Yellow Globe Danvers onion seeds were surface-sterilized and germinated under sterile conditions on potato-dextrose agar. After 6 to 8 days, seedlings in plates showing neither fungal nor bacterial growth were transferred to Petri dishes containing 14-day-old cultures of a recent isolate of *P. terrestris*. Care was taken to place the seedlings at the edges of the cultures, which were then incubated at 25° C. The seedlings were removed at 12-hour intervals, cut into small pieces, killed, and fixed. A second lot of sterile seedlings was removed and planted in steamed soil into which the pathogen had been introduced. These seedlings were removed at 7-day intervals, cut into small pieces, killed, and fixed. The material was then cleared, embedded in paraffin, sectioned, and stained, using standard procedures.

The Mechanism of Invasion.—Onion roots grown in artificially infested soil and those placed adjacent to the edges of cultures of the pathogen showed the same general type of invasion. The fungus first appeared on the surface of young roots as small, irregular, colonies. Such growths were

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seen readily when the roots were cleaned in water, stained in hot cotton-blue lacto-phenol solution, mounted and examined microscopically in the uncrushed condition (Fig. 1, A). The hyphae of the pathogen stained a deep blue, leaving the surface of the root relatively colorless. Primary-invasion hyphae were observed as a result of the deep stain taken by the external hyphal segment, in contrast with the lack of stain in that portion of the same segment within the epidermal cells.

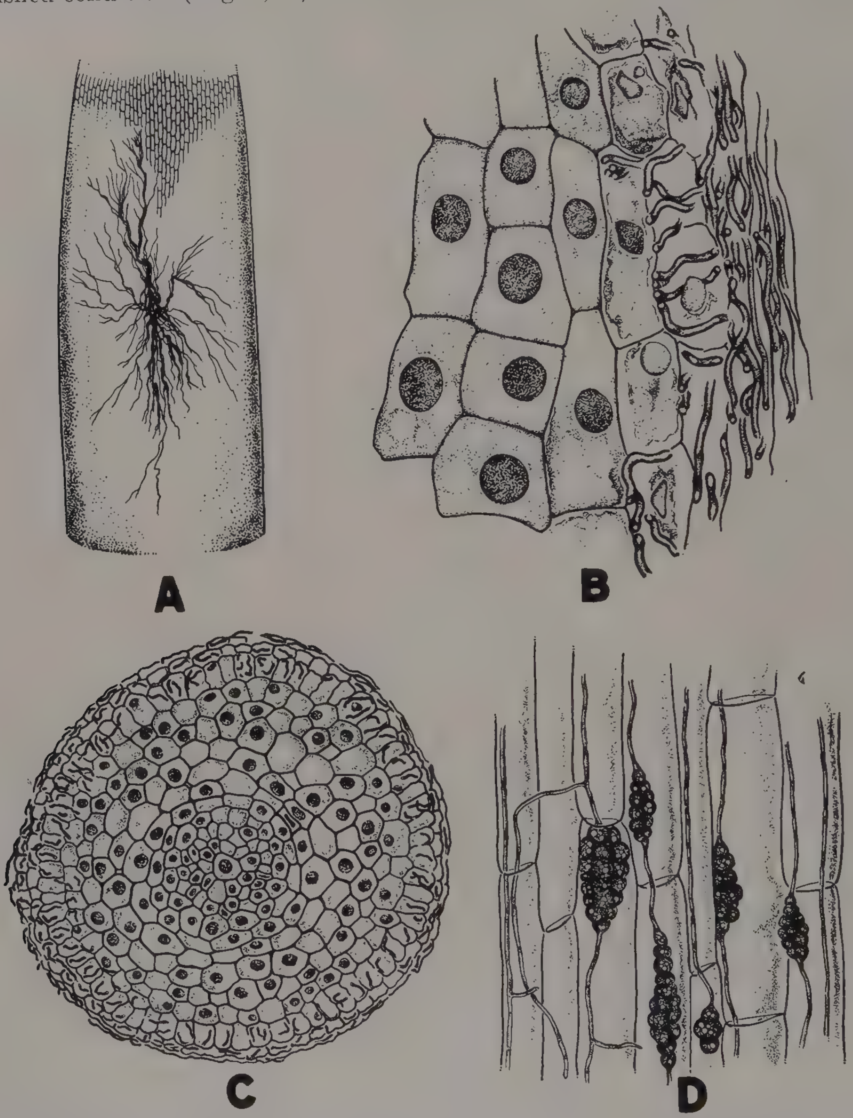

FIG. 1. Invasion of the onion root by *Phoma terrestris*. A. Initial stage showing a small, colony-like growth of the fungus on the surface of a young root. B. The invasion of cells adjoining the promeristematic region of the root. Plasmolysis and nuclear distortion were characteristic phenomena observed in such affected tissue. C. Transverse section of a root just back of the promeristem showing early cortical invasion. D. The pycnidial primordia of *P. terrestris* in the cortical cells of an infected onion root.

After entering the young root, the fungal hyphae proceeded in all directions from the point of primary invasion, ramifying throughout the cortex, intra- and intercellularly. Examination of prepared root sections revealed cases of hyphal constriction where the walls of the cortical cells were penetrated (Fig. 2, C). Pigmentation usually was confined to the hyphae of the pathogen (4), although observations frequently revealed that there had been some apparent diffusion of the coloring substance into invaded cells. The pigment was not manifested in non-invaded cells. The extensive diffusion of pigment mentioned by Hansen (3) was not observed. Generally, in cases of artificial inoculation, the fungus appeared to sweep across the

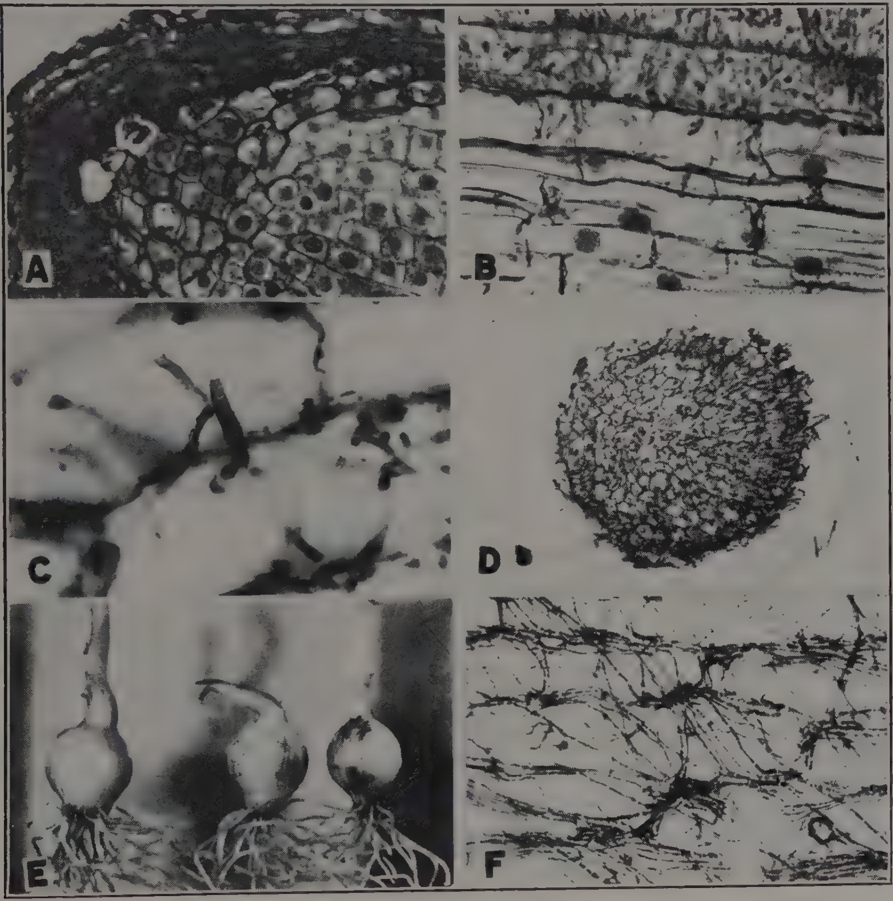

FIG. 2. Invasion of the onion root and outer bulb-scale tissue by *Phoma terrestris*. A. Longitudinal section showing the affected root cap; the darker areas are filled with the stained hyphae of the pathogen. The promeristem shows no invasion. B. Longitudinal section showing hyphae advancing through the cortex of the root. C. Hyphal constriction at the point of penetration of a cell wall in the cortex of the root. D. Transverse section illustrating the final stage in the root showing the completely invaded and necrotic tissues. E. Blemish of the outer bulb scales of white onion (variety Southport White Globe) is shown by the two plants on the right. F. The invaded outer scale tissue of the onion bulb, showing the profuse stained mycelium of the pink-root organism.

cortex in a hyphal mass (Fig. 1, C and Fig. 2, B). Under such conditions the root soon collapsed (Fig. 2, D).

Invasion of Onion Root Tips.—Since protoplasmic responses to invasion in the cells of the cortex could not be ascertained with any degree of certainty, the reaction of the meristematic and root-cap cells was studied. Microscopic examination of prepared root-tip sections revealed a condition such as that shown in figures 1, B and 2, A. As shown by the former figure, the invaded root-cap tissues soon collapsed, showing only the vestiges of distorted cell walls. Cells into which fewer hyphal segments penetrated revealed plasmolysis of their contents and distortion of the nuclei, which latter frequently failed to take or to retain a safranin stain. Cells adjacent to those invaded, but apparently not parasitized, showed slight plasmolysis. In no case was the promeristematic region invaded.

Pycnidial Primordia.—These bodies were first seen by Hansen (3), who described their occurrence in onion-root tissue invaded by *Phoma terrestris* and in cultures of the fungus growing on cornmeal agar. Hansen considered them to represent an early stage in the formation of pycnidia. Microscopic examinations of pinkened onion roots obtained from naturally and artificially infected plants have revealed the invariable presence of pycnidial primordia in the cortical and epidermal tissues (Fig. 1, D). Such observations have been verified by isolations. These bodies were, moreover, always present in yellow and yellow-brown roots, infected by *Phoma terrestris* (4). Although pycnidial primordia almost invariably appeared in 30- to 90-day-old stock cultures of the organism growing on potato-dextrose agar, in the case of only one isolate were they observed to develop into pycnidia.

The Action of the Pathogen on Onion Bulbs

As noted by Hansen (3) and Davis and Henderson (1), the pink-root fungus did not invade the living tissues of the onion bulb. Numerous trials were conducted in which bulbs of standard varieties, such as the Yellow Globe Danvers, Red Wethersfield, and Southport White Globe, were inoculated with *Phoma terrestris*. In no instance was any invasion of inoculated bulbs by the fungus observed. However, when sets or mature onion bulbs of varieties, such as the Silverskin or Southport White Globe, were grown in soils previously infested with the pink-root fungus, it was observed that they showed pink to purple-red outer scale blemishes 7 to 21 days following planting (Fig. 2, E). Bulbs grown in noninfested soils did not show this condition. Microscopic examinations of portions of such discolored tissues revealed the presence of fine, intricately branching hyphae (Fig. 2, F), and isolations from such areas yielded the pink-root fungus in the majority of instances. In yellow or red varieties, such as the Yellow Globe Danvers or Red Wethersfield, invasion was manifested by the appearance of water-soaked areas on the outer scales. Examinations of numerous commercial lots of set bulbs or mature onion bulbs almost invariably showed a number of bulbs thus affected. The pink-root fungus has been isolated in several instances from the discolored scales of white onion bulbs taken directly from the field. In addition, examination of such affected tissues usually revealed the presence of pycnidial primordia. No invasion from the affected dead outer scales into the living leaf tissue of the bulb was observed.

Fungi mentioned in the literature as being capable of attacking the scales of onion bulbs producing unsightly blemishes are *Macrosporium porri* Ell., *M. parasiticum* Thüm. (8), *Colletotrichum circinans* (Berk.) Voglino (9), and *Phoma alliicola* Thüm. (7, 9). The last named fungus, according to Walker (9), "produces small black pycnidia which are often difficult to distinguish macroscopically from the stromata of the smudge fungus (*Colletotrichum circinans*)." There is apparently no evidence of the effect that *P. alliicola* will invade onion roots. In addition no true pycnidia were found during the present study on scale tissues attacked by *Phoma terrestris.*

Effect of Pink Root on the Incidence of a Bulb Decay Caused by Fusarium vasinfectum var. zonatum

Davis and Henderson (1), in studying the relationship between a basal bulb-rot of onions caused by *Fusarium vasinfectum* var. *zonatum* f. 1. and pink root, reported that the bulb-rotting pathogen caused semi-dry rot of onion bulbs in the field and in storage. It did not, however, attack the roots or bulbs, except following injury or initial invasion by *Phoma terrestris.*

In order to ascertain whether root attack by *Phoma terrestris* would affect the incidence of bulb-rot induced by local bulb-rotting isolates of *Fusarium*, a study was conducted with 3 isolates which were identified as cultures of *Fusarium vasinfectum* var. *zonatum* and had been demonstrated to be quite pathogenic to injured onion bulbs, and 5 other unidentified cultures of *Fusarium* which had been obtained from onion roots showing pink root, but had been found to be only weakly pathogenic to injured onion bulbs. Since it had been observed that cultures of *P. terrestris* obtained from various localities in the State frequently differed considerably in virulence, one of the more virulent of these isolates was used in the study.

Large Southport White Globe onion-set bulbs were selected for both size and appearance, washed, treated with a 0.2 per cent solution of $HgCl_2$ for 15 minutes, and then rinsed with sterile water. Two hundred and seventy bulbs were selected and individually injured by thrusting a sterile needle through the stem. Two similar lots were kept free from injury. The lot of injured bulbs was planted deeply, 3 to a container, in steamed 4-in. pots containing steamed soils infested singly with all the different isolates of *Fusarium*. A lot of uninjured bulbs was treated in the same fashion. The second lot of uninjured bulbs was planted in a like manner in pots containing soils infested with one of the following organisms or combinations of organisms: *Phoma terrestris*, *P. terrestris* + *Fusarium vasinfectum* var. *zonatum*, and *P. terrestris* + *F. vasinfectum* var. *zonatum* + one of the unidentified isolates of *Fusarium*. A total of 8 g. of inoculum was used for each soil infestation. Observations were made at the end of a 70-day period.

The results of this study showed that the isolates of *Fusarium*, especially cultures of *Fusarium vasinfectum* var. *zonatum*, caused bulb rot only where the bulb was mechanically injured. Such results were in accordance with earlier trials by the writer, and also with those obtained by Walker and Tims (10), and Link and Bailey (5), who reported that the onion bulb-rotting species of *Fusarium* they studied were wound parasites and apparently did not attack uninjured bulbs. In addition root invasion by the isolate of *Phoma terrestris* apparently did not afford an entrance into the bulbs for the isolates of *Fusarium,* since bulb-rot was observed in no case in the mixed infestation series. Severe pink root occurred in all instances where *P. terrestris* was introduced into the soil. Other studies such as the one described gave essentially the same results.

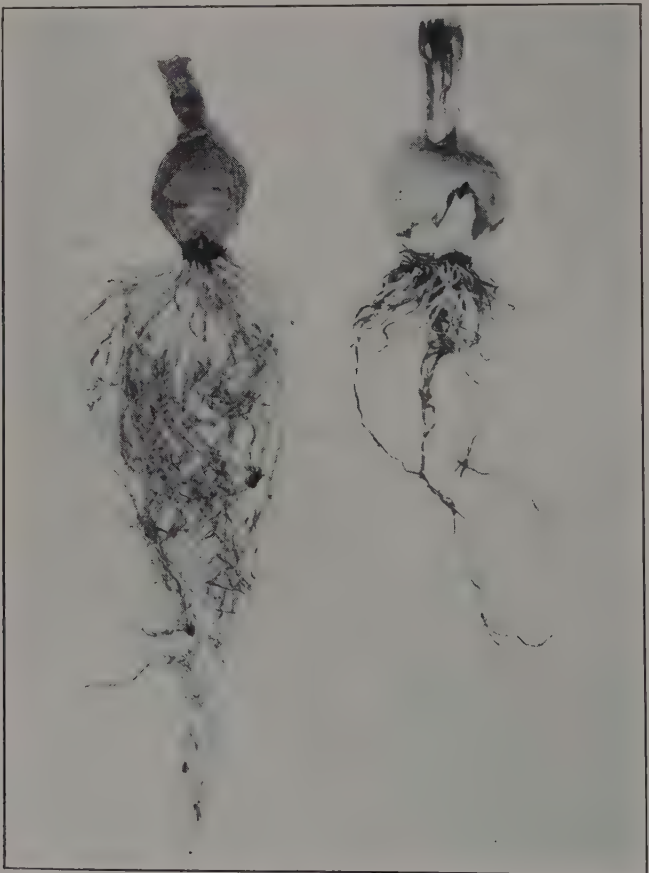

FIG. 3. Infection of the onion by *Phoma terrestris* and *Fusarium vasinfectum* v. *zonatum*. Left, the decaying bulb and apparently healthy root system of a plant developed from an injured bulb planted in soil infested with *F. vasinfectum* v. *zonatum*. Right, severe pink root and scale blemish on a plant developed from an uninjured bulb planted in soil infested with cultures of both *F. vasinfectum* v. *zonatum* and *P. terrestris*.

Figure 3, left, shows the effect of planting an injured bulb in soil infested with *Fusarium vasinfectum* var. *zonatum*. It will be observed that, although the bulb was almost completely decayed, the roots were unaffected. In figure 3, right, is shown the result of planting an uninjured bulb in soil infested with cultures of *P. terrestris* and *F. vasinfectum* var. *zonatum*. Scale invasion and root attack by the pink-root pathogen are evident, but the bulb is unaffected.

The Action of Phoma terrestris on Hosts Other Than Allium cepa

In studying the resistance of some of the cultivated species of *Allium* to *Phoma terrestris,* Porter and Jones (6) found that, although garlic, *A. sativum* L., and shallot, *A. ascalonicum* L., were susceptible, the Nebuka onion, *A. fistulosum* L., leek, *A. porrum* L., and chives, *A. schoenoprasum* L., were extremely resistant.

In 1929 Hansen (3) reported that he had isolated *Phoma terrestris* from potato tubers, *Solanum tuberosum* L., and from lesions on the roots of cow pea, *Vigna catjang* Walp., and Lima bean, *Phaseolus lunatus* L.

In order to investigate the probability of other common crop plants being attacked by *Phoma terrestris*, a study was undertaken that was confined to the types of standard field crops which might be expected to precede or to follow onion plantings. A soil mixture (1 part sifted sheep manure, 1 part sand, and 4 parts loam) was steamed for 3 hr. at 17 lb. pressure and placed in 15 well-washed flats and allowed to cool. Into the soil of each of 10 of these flats 40 g. of ground wheat inoculum (45-day-old culture of *Phoma terrestris*) was introduced. As a control measure 40 g. of ground steamed wheat was added to the soil in each of the 5 remaining flats. The ground inoculum and steamed wheat were mixed thoroughly into the soil, and the flats were then allowed to stand for a few days before planting. At the end of this time seeds of 28 crop plants other than onion were planted in the flats in rows. Between each of these rows a row of Mountain Danvers, Red Wethersfield, or Riverside Sweet Spanish onion seed was planted as a control. Observations and isolations were made 30 days following planting. This test was repeated, employing the same methods and varieties of seeds.

Phoma terrestris attacked the roots of seedlings of 19 different crop plants. Pink root was severe or moderate on Mountain Danvers and Red Wethersfield onion; Black Amber cane sorghum, *Sorghum vulgare* var. *saccharatum* (L.) Boerl.; Early Fortune millet, *Panicum miliaceum* L.; Davis Perfect cucumber, *Cucumis sativus* L.; Oxheart carrot, *Daucus carota* L.; and spinach, *Spinacia oleracea* L. Light root attack was observed on Edwards pea, *Pisum sativum* L.; Brunker oats, *Avena sativa* L.; Trebi barley, *Hordeum vulgare* L.; Komar wheat, *Triticum aestivum* L.; Improved Golden Bantam sweet corn, *Zea mays* L. var. *saccharata* Sturt.; Minnesota 13 dent corn, *Zea mays* L. var. *indentata* Sturt.; Hubbard squash, *Cucurbita* *maxima* Duc.; Golden Honeymoon cantaloupe, *Cucumis melo* L. var. *reticulatus* Naud.; Extra Early Osage muskmelon, *Cucumis melo* L. var. *reticulatus* Naud.; John Baer tomato, *Lycopersicum esculentum* Mill.; New York Improved eggplant, *Solanum melongena* L. var. *esculentum* Nees; and Super Snowball cauliflower, *Brassica oleracea* L. var. *botrytis* DC. A light attack was observed also on the Sweet Spanish onion. This variety has been reported by Porter and Jones (6) as less susceptible to attack by *Phoma terrestris* than other horticultural varieties. Those plants showing no symptoms of pink root were Giant Musselberg leek, *Allium porrum* L.; soybean, *Glycine hispida* Maxim.; Pinto bean, *Phaseolus vulgaris* L.; red clover, *Trifolium pratense* L.; sweet clover, *Melilotus alba* Desv.; alfalfa, *Medicago sativa* L.; Oshkosh and World Beater pepper, *Capsicum annuum* L.; Stem Holland cabbage, *Brassica oleracea* L. var. *capitata* DC.; Hollow Crown parsnip, *Pastinaca sativa* L.; Early Blanching celery, *Apium graveolens* L.; Pioneer sugar beet, *Beta vulgaris* L.; Black Seeded Simpson lettuce, *Lactuca sativa* L.; and Earliana tomato.

Although showing no characteristic pink-root symptoms, tissue plantings from the water-soaked roots of World Beater pepper and soybean on nutrient agar, yielded cultures of *Phoma terrestris*. The pink-root organism was isolated from the roots of onion (all varieties used), soybean, millet, oats, John Baer tomato, World Beater pepper, carrot, and spinach. The fungus was obtained also from the pericarps of germinating caryopses of barley, wheat, and corn (both varieties used). The control plants growing in flats containing the steamed, noninfested soil were healthy.

SUMMARY

Phoma terrestris, the causal agent of pink root of onions, formed small, colony-like growths on onion roots; invasion was effected by means of hyphae showing characteristic constrictions at the point of entrance. Having once gained access to the inner tissues, the hyphae ramified throughout the cortex, eventually forming the cellular agglomerations known as pycnidial primordia in the cortical and epidermal cells. Under conditions favorable for the growth of the organism affected roots then underwent complete necrosis. Invaded cells near the promeristematic region revealed plasmolysis of their contents and nuclear distortion. The nuclei usually failed also to take or retain a safranin stain. Cells showing no invasion, but adjoining those containing the hyphae of the pathogen, revealed a slight plasmolysis of their contents.

Although the pink-root pathogen did not invade the living leaf tissue of the onion bulb, it invariably did attack the dead outer scale tissue of the bulb. Such invasion, because of the typical reddish discolorations produced, was more evident in the case of white onions than in that of the colored varieties.

No evidence was obtained to the effect that root attack by *P. terrestris* enabled isolates of the bulb-rotting pathogen, *Fusarium vasinfectum* var.

zonatum, to invade and rot previously uninjured bulbs. Isolates of the latter fungus readily attacked injured bulbs but failed to affect them when they were not injured.

Phoma terrestris attacked the roots of numerous young crop plants other than onion. Plants shown to be susceptible to attack were soybean, pea, cane, millet, oats, barley, wheat, corn, squash, cucumber, cantaloupe, muskmelon, tomato, pepper, eggplant, cauliflower, carrot, and spinach.

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LITERATURE CITED

1. Davis, Glen N., and W. J. Henderson. The interrelation of the pathogenicity of a *Phoma* and a *Fusarium* on onions. Phytopath. **27**: 763–772. 1937.
2. Hansen, H. N. Pink root of onions caused by *Phoma* sp. Science (n. s.) **64**: 525. 1926.
3. ———. Etiology of the pink-root disease of onions. Phytopath. **19**: 691–704. 1929.
4. Kreutzer, W. A. Host-parasite relationships in pink root of *Allium cepa* L. I. The pigment of *Phoma terrestris.* Phytopath. **29**: 629–632. 1939.
5. Link, George K. K., and Alice A. Bailey. Fusaria causing bulb rot of onions. Jour. Agr. Res. [U. S.] **33**: 929–952. 1926.
6. Porter, D. R., and H. A. Jones. Resistance of some of the cultivated species of *Allium* to pink root (*Phoma terrestris*). Phytopath. **23**: 290–298. 1933.
7. Saccardo, P. A. Sylloge fungorum **3**: 157–158. Patavii. 1884.
8. Thaxter, Roland. Report of Roland Thaxter, Mycologist. Conn. Agr. Exp. Stat. Ann. Rpt. **1889**: 127–177. 1890.
9. Walker, J. C. Onion smudge. Jour. Agr. Res. [U. S.] **20**: 685–721. 1921.
10. ———, and E. C. Tims. A fusarium bulb rot of onion and the relation of environment to its development. Jour. Agr. Res. [U. S.] **28**: 683–694. 1924.

THE BUCKSKIN DISEASE OF CHERRY AND OTHER STONE FRUITS

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Evidence was presented in a previous publication (5) indicating that the virus causing the buckskin disease of sweet cherry (*Prunus avium*) in California also causes a destructive disease of peach (*P. persica*). The symptoms produced by this virus on peach and western chokecherry (*Prunus demissa*) are very similar to, if not identical with, those reported in other States on infected peach and eastern chokecherry (*Prunus virginiana*), where the disease has been called X-disease or yellow-red virosis. Since it apparently is becoming one of the most important viroses of stone fruits, it appears desirable to present at this time additional information regarding the disease in California.

PREVALENCE

The disease in cultivated sweet cherry is known in Contra Costa, Napa, Solano, and Sonoma Counties. Thus far the peach orchards in the State with any considerable number of infected trees are rather consistently associated with affected cherry orchards. No extensive infections, however, have thus far been found on peach in the vicinity of the severely affected cherry orchards of the Napa area; as will be shown later, the disease in Napa appears to be distinct in several ways from the type prevalent on cherry and peach in Green Valley, Solano County. A few infected peach trees have been seen in Santa Clara and Merced Counties at some distance from known cherry trees, cultivated or wild. No natural infections have yet been found in the State on *Prunus demissa;* this species has not been found near affected orchards. Although the virus causing this disease, or closely related strains, is widely distributed in the cooler States, it does not seem to be prevalent in the warmer areas. It is of special interest, therefore, to know whether the virus is able to persist or produce symptoms in the warm interior valleys of California. Seemingly typical symptoms have recently been observed in several trees of the Phillips Cling peach at Merced, in the relatively hot San Joaquin Valley. Cions, moved to Berkeley and grown in the greenhouse, have exhibited typical symptoms.

SYMPTOMS

The symptoms of the disease of sweet cherry described in earlier papers (2, 3) were those observed primarily in Green Valley, Solano County, California. It has been apparent, however, for several years that the symptoms in certain orchards of adjacent Napa County are distinct. Since the evidence to be presented indicates that the Green Valley and Napa viruses are distinct strains, the symptoms observed in each district will be treated in some detail.

Symptoms on Sweet Cherry (Prunus avium)

Certain branches of trees on Mazzard (*P. avium*) stocks bear fruits that are small and fail to mature. In Green Valley these fruits are conical and persistent, with short, thick pedicels; the surface of the blossom-end half of the fruits assumes a dull or ''buckskin'' aspect, especially noticeable on varieties with light colored fruits (Fig. 1, B). Comparable small affected fruits in Napa Valley are nearly normal in shape and usually lack the dull surface; the pedicels are of normal length and easily detached from the tree (Fig. 1, A, F). Affected spurs in Napa bear ragged buds as shown in figure 1, C; a comparable healthy spur is shown at figure 1, I. This symptom has not been observed in Green Valley.

In trees on *Prunus mahaleb* stocks, most fruits are approximately normal in appearance (Fig. 1, D), but have short pedicels, are soft and insipid, and ripen early. In Napa Valley occasional small branches may produce undersize fruits that fail to ripen. In Green Valley some fruits may be larger than normal, conical in form, and with very thick pedicels.

Young cherry trees on Mazzard stocks usually do not exhibit symptoms during the first season after infection and, except in fruit, older trees in Green Valley seem approximately normal till near the end of the season. Then some leaves often become bright orange-red along the basal part of the midvein and in the adjacent lamina. This symptom was illustrated earlier (2). Older trees on this stock in Napa Valley develop the late-season coloration in the basal part of the leaf, but, in advanced infections, the fruit spurs make a terminal growth and produce an excess of small pale green leaves (Fig. 1, F). A comparable healthy branch is shown in figure 1, E. In both valleys infected trees on Mazzard stocks, unlike those on Mahaleb, usually live for many years.

Young trees on Mahaleb stocks usually die in the first year after infection, sometimes quite suddenly, with the dried leaves persisting, but usually more slowly, accompanied by severe general chlorosis, cupping, and premature dropping of leaves. The leaves often develop an orange color along the midrib and lateral veins just before abscission. Old trees on Mahaleb stocks in Green Valley show the same symptoms. Older trees on Mahaleb in Napa Valley have sparse small pale green leaves and many dead or missing spurs (Fig. 1, D). Such trees usually die within 2 or 3 years after infection.

Symptoms on Peach

The principal symptoms on peach in the orchard have been described by several observers (4, 5). Apart from slight delay in blossoming, distinctive symptoms are not seen till May or June. At this time the growth of older affected branches is much retarded while other branches of the same tree may appear to be normal. The leaves on such affected branches are usually yellowish-green, rolled, and recurved toward the stems, but may or may not show other symptoms. Leaves on the younger and more vigorous affected

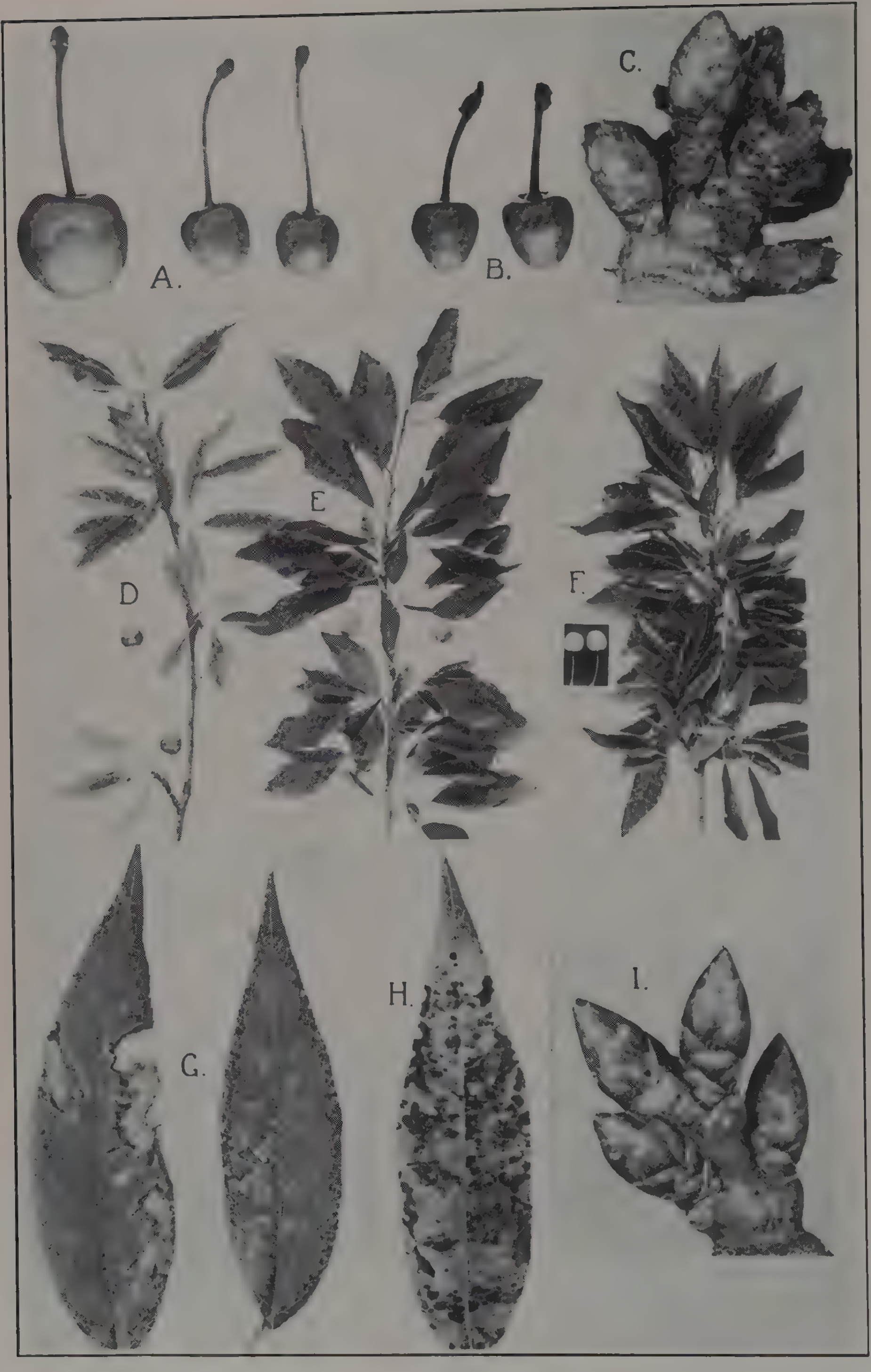

FIG. 1. For legend see page 919.

shoots, after reaching approximately mature size, develop slight to marked chlorosis and rolling and few to many chlorotic, reddish or purplish blotches of indefinite margin. These symptoms are accompanied or followed by abscission and death of parts of the lamina, resulting in a very ragged appearance. The abscissed parts are typically rounded in outline but in one seedling type of peach may be almost linear and closely associated with the larger veins. Finally the leaves drop, beginning with the oldest and often leaving the affected shoots bare by midsummer. Fruits shrivel and drop at various stages up to about half that of full maturity.

The red or purple coloration may vary in degree between wide extremes, depending on the variety, the stock, state of vigor, and probably other factors (Fig. 1, G, H).

Early symptoms on vigorous plants in the greenhouse resemble those in the orchard. In extremely severe cases, areas in the bark of new shoots, as well as in the leaf blade, may be killed. Plants in low vigor or inoculated late in the growth cycle, on the other hand, often develop only small leaves, general yellowing, rolling, and vein swelling.

Symptoms on Sour Cherry (Prunus cerasus)

English Morello trees on Mazzard stocks, inoculated with buckskin Napoleon cions from Green Valley, produced little terminal growth. The fruits remained small, and shrivelled before ripening. Healthy Napoleon cions, grafted on such trees the year following inoculation, developed typical buckskin symptoms. Young seedlings of English Morello, inoculated from peach in the greenhouse, developed premature general yellowing of leaves with small green islands persisting. Such leaves dropped soon after the chlorosis appeared.

Symptoms on Prunus mahaleb

In previous orchard experiments this species appeared to be immune (3). Recent inoculations of greenhouse plants indicate that Mahaleb can be infected and the virus can be recovered from branches that show symptoms. The symptoms are seen on a few branches near the points of inoculation. Such branches make little or no growth; internodes may be shortened and leaves may become light-green and much reduced in size. Relatively few of the inoculated Mahaleb trees have shown symptoms.

FIG. 1. Symptoms of buckskin disease. A. Healthy Napoleon fruit on left, and at right 2 diseased Napoleon fruits from tree on Mazzard stock, Napa Valley. B. Buckskin Napoleon fruits from tree on Mazzard stock, Green Valley. C. Ragged buds from diseased Napoleon tree on Mazzard stock, Napa. D. Buckskin Napoleon branch from tree on Mahaleb stock, Napa. E. Branch from healthy Napoleon tree. F. Buckskin Napoleon branch from tree on Mazzard stock, Napa. G and H. Variation in symptoms exhibited by infected peach leaves on cions originally from the same infected tree. The two leaves on the left were grown on an almond stock of low vigor and show little change in color other than the chlorosis in areas that are to drop out. The leaf on the right was grown on a vigorous Myrobalan and shows distinct red to purple blotches. I. Spur from healthy Napoleon cherry tree comparable with diseased spur shown in C.

Symptoms on Other Species

Symptoms in the greenhouse-inoculated plants of *Prunus communis, P. mira* and several hybrids of these with *P. davidiana, P. fenzliana* and *P. persica* are, on the whole, less specific and distinct than those on peach; necrotic areas are small or sometimes lacking and when present are often oblong to linear along veins, rather than rounded. Often there is no more than a general yellowing of parts or all of the leaf blade and an upward rolling of leaf edges, usually accompanied by vein swelling. The leaves may drop early or persist through the season. In one peach-almond hybrid tested there is a marked shortening of internodes but not in another. No symptoms have been recognized on apricot, though the virus was recovered from or passed through Blenheim apricot growing on diseased peach by inarching a healthy peach seedling on the apricot. There was pronounced swelling at the nodes of the apricot, but this may have been due to the relatively unfavorable stock.

EVIDENCE OF TWO STRAINS OF THE VIRUS

The differences in symptoms on cherries between Green Valley and Napa, even when the same variety and stock are studied, suggest differences in the virus. Among the more obvious differences in symptoms are, in Green Valley: apparently normal flower buds, conical dull-surface fruits, early and midseason foliage approximately normal if on Mazzard stock, severely chlorotic on Mahaleb; in Napa Valley: ragged flower buds, normal-shape fruits without distinctly dull surface, leaves of older trees small and pale green on both stocks, numerous on Mazzard and sparse on Mahaleb.

More direct evidence is provided by a test in which affected Napa cions were grafted on 4 healthy cherry trees on Mazzard stocks in an experimental plot in Green Valley. The symptoms on affected fruits on the inoculated trees were essentially those in Napa Valley, whereas fruits in the same plot on trees infected from local material showed symptoms typical of Green Valley.

In general, throughout California, there is considerable variation in the symptoms on peach, but the influence of variety and environment on this variation has not been sufficiently studied.

INFECTION EXPERIMENTS

Marked differences in effectiveness as inoculum have been noted between varieties of cherries (2), varieties of peaches (5) and between the most effective cherry variety (Napoleon) and the best peach variety tested thus far (Orange Cling). It is now known also that the Napoleon cherry may be a good source of inoculum if on Mazzard stock and extremely poor when on Mahaleb. A better peach source is still needed, and recent tests suggest that it may be found among peaches from Fukien, China, now being bred in California because of their low winter-chilling requirement.

First symptoms appear commonly below points of inoculation. Preliminary tests suggest that the virus moves more readily into defoliated branches than into those with leaves, but the results have not been entirely consistent, perhaps partly because of the tendency of defoliated branches to cease growth soon after defoliation.

The incubation period in the field varies from several months to several years. On small plants in the greenhouse a period of about 2 months usually is required for the appearance of symptoms. In a single instance early symptoms were seen on peach 33 days after inoculation.

Some of the results of recent inoculations are shown in table 1. The cherry inoculum (cions containing Green Valley virus) was taken in all cases from Napoleon on Mazzard stock, and the inoculated sweet cherries were Napoleon on Mahaleb, except in the orchard-grown lot in which the trees were on Mazzard. Peach inoculum was taken from several sources including Elberta, Orange Cling and seedlings. Inoculum of *Prunus demissa* was obtained from plants infected by grafting from sweet cherry. The first lot of each of the inoculated sour and sweet cherries was grown in the field in Green Valley. All the other plants were kept after inoculation in greenhouses. With the exceptions noted in the table footnotes, and a few others in which inarching was used, plants were inoculated with one infected cion per tree.

TABLE 1.—*Results of graft inoculations with buckskin virus*

Inoculum	Plant inoculated			Approximate duration of test-months
	Name	Number inoculated	Number infected	
P. avium	*P. persica*[a]	6	3	12
P. avium	*P. persica*[b]	5	4	8
P. avium	*P. persica*[c]	5	1	8
P. avium	*P. cerasus*[d]	7	6	16
P. demissa	*P. avium*	14	6	8
P. demissa	*P. persica*[b]	10	4	9
P. persica	*P. avium*	10[e]	2	7
P. persica	*P. avium*	10[f]	0	8
P. persica	*P. avium*	10[g]	1	8
P. persica	*P. cerasus*[h]	18	3	9
P. persica	*P. demissa*	18	0	9
P. persica	*P. mahaleb*	20	3	8
P. persica	*P. persica*[a]	10	3	8

[a] Seedlings, [b] Seller's Orange, [c] Elberta, [d] English Morello on Mazzard, [e] Five to 7 cions per tree, [f] One cion per tree, [g] Three cions per tree, [h] Morello seedlings.

As shown in the table distinct difference in susceptibility was found between Elberta and Sellers Orange peaches. Symptoms appeared on Sellers Orange 2 months after inoculation, but were not seen on Elberta till 6 months later. Peach was inferior to sweet cherry and chokecherry as a source of inoculum, even when peaches were inoculated. It is, therefore, rather surprising that any of the resistant *Prunus mahaleb* were infected by peach cions. The virus has, however, been recovered from at least 2 of them by inarching to peach seedlings. It apparently moves very slowly in plants of this species, since branches more than a few inches from the point of inoculation continue to grow normally up to 10 or more months after inoculation.

A number of inoculations were made on plants not mentioned in the table. The Shalil peach is readily infected, with symptoms similar to those of the more common varieties.

Among more than 40 apricot trees inoculated from peach a few developed small leaves and short internodes and eventually died, but attempts to recover the virus from them failed. It was, however, obtained from a Blenheim apricot top, growing on an infected peach tree, by inarching a healthy peach tree on the apricot.

Symptoms on almond are often so indefinite as to preclude certain diagnosis. However, several of the 40-odd inoculated plants developed symptoms that seemed to result from the inoculation; the virus was recovered from at least one of them. Plants of each of 2 peach × almond hybrids were infected by inoculation from peach. Almonds, apricots, and various hybrids more closely related to peach are of interest, among other things, as possible resistant stocks on which peach might be grown as is done in the case of sweet cherry on the resistant Mahaleb stock (3). The results with the possible peach stocks have, to date, not been encouraging. In addition to the above at least some susceptible individuals have been found among hybrids of *P. mira* × *P. persica, P. davidiana* × *P. communis* and *P. communis* × *P. fenzliana.*

Prunus mira was infected by inoculation and developed definite but mild symptoms.

Preliminary attempts have not produced symptoms in the following: *Prunus andersoni, P. cerasifera, P. davidiana, P. domestica* (French prune), *P. emarginata, P. fenzliana, P. fremontii, P. ilicifolia, P. marianna, P. subcordata* and *Rosa californica.* Likewise, attempts to recover the virus from inoculated plants of *P. cerasifera, P. emarginata, P. ilicifolia, P. marianna* and *P. sub-cordata* have been unsuccessful. No symptoms have been seen on any plants of the plum and prune group, whether artificially inoculated or exposed to natural inoculation. Attempts to recover the virus from varieties of *P. domestica* have not been concluded.

HISTOLOGY AND MICROCHEMISTRY[1]

Evidence of degeneration in the phloem tissues is readily seen in transverse sections of affected peach stems. The affected cells, typically midway

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[1] The writers are indebted to Mr. Henry Schneider for assistance in histological and photographic work.

FIG. 2. Histological abnormalities in infected tissues. A. Cross section of swollen vein of infected peach leaf, showing cavities on each side of the vascular bundle and hypertrophied cells growing into the cavity on the right. B. Portion of a cross section of an infected peach stem showing degeneration in the phloem revealed by staining with phloroglucinol. C. Cross section through the narrow abscission zone that separates the chlorotic region of a peach leaf from the surrounding green tissues, showing separation of cell walls due to solution of intercellular substance (indicated by arrows).

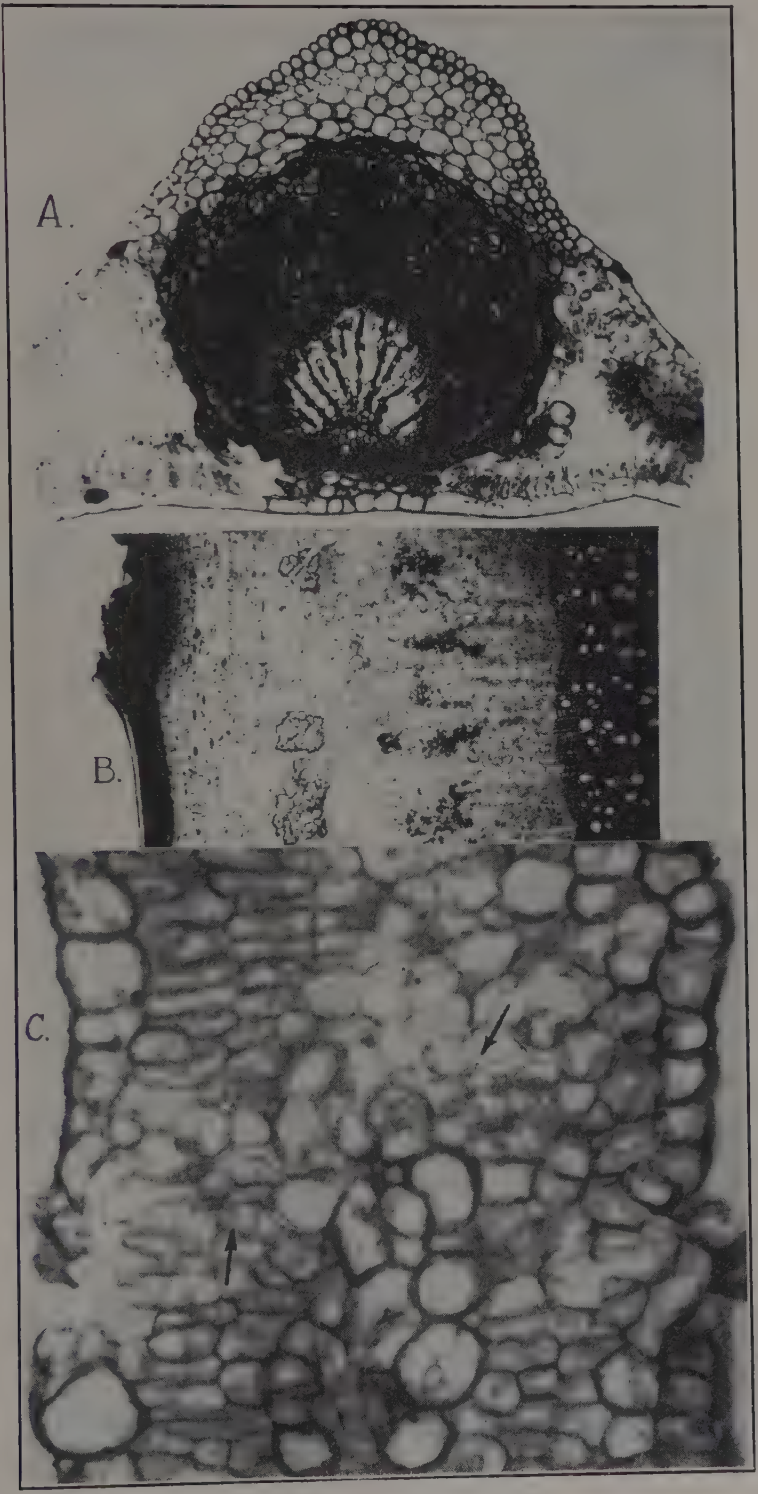

FIG. 2. For legend see page 922.

between the phloem rays, stand out in sharp contrast with other cells nearby when stained red by phloroglucinol in 18 per cent hydrochloric acid (Fig. 2, B). In stems produced during the season after inoculation, the outer (older) phloem region usually is most affected, although in severe cases the injury may extend inward radially to near the cambium. Stems that have grown a season before and a season after inoculation exhibit greatest injury about midway radially in the phloem region (Fig. 2, B).

The phloem of cherry stems from the upper portion of infected young trees on Mahaleb stocks gives a distinct reaction with phloroglucinol, but less striking than that in peach. When sections, however, are taken from the base of the main stem a severe breakdown in the phloem is observed.

The swollen veins, commonly seen on infected peach leaves grown under glass, are characterized in surface view by narrow ridges on each side of the vein on either leaf surface. Beneath these ridges are cavities of considerable size at the junction of the lamina and the vein. No evidence of necrosis is seen. Viewed in section (Fig. 2, A), the separation of cells to produce the cavity seems to be due chiefly to enlargement of the vascular bundle by hypertrophy and also by hyperplasia. Several types of cells are involved in the enlargements, including those of the phloem rays, phloem parenchyma, and the bundle sheath. Giant cells, arising in some cases in or near the bundle sheath, may grow out into the cavities in late stages (Fig. 2, A). Many of the affected cells in the phloem rays and bundle sheath in sections fixed in chromic fixing solutions appear completely filled with a brown or yellow substance. Such cells in sections fixed in 8 per cent formalin and treated with 10 per cent ferric chloride become a dense black, indicating the presence of phenolic constituents. Vein swelling is not peculiar to this disease, and a sufficient study has not been made to differentiate the changes noted above from those produced by other causes.

One of the most characteristic and unusual symptoms of the disease on peach leaves is the abscission along the margin of the chlorotic or purple areas. This abscission frequently precedes necrosis of the areas, as is shown in the upper left portion of the leaf at right in figure 1, G. A microchemical study of this phenomenon yielded some very interesting results. Among the first histological abnormalities observed in the chlorotic or purple areas is the appearance of numerous spheroid bodies of various sizes within the cells in the discolored portion of the leaf. These bodies become black when treated with 8 per cent formalin followed by 10 per cent ferric chloride solution. This reaction indicates that they are composed of tannin or contain phenolic constituents.

In a narrow abscission zone surrounding these areas the contents of the cells are dissolved, as shown most clearly in the palisade parenchyma in the middle of the section in figure 2, C. Of most interest is the behavior of the intercellular substance between the walls of the cells in this zone. The cell walls are stained with ruthenium red but the intercellular substance between the walls in the abscission zone has been dissolved and the walls are pulled apart (Fig. 2, C). This early solution of the intercellular substance evidently is responsible for the early abscission along the margin of the degenerating leaf area before necrosis of the area has occurred.

In the leaf tissues just outside of the abscissive zone, wound gum is formed and suberization finally develops, as has been reported by Hewitt (1) in his careful study of abscission in the petiole of the olive.

SUMMARY

Symptoms of the buckskin disease on cherry, peach, and certain other stone fruits are described. The rootstock has a marked effect on the symptoms of sweet cherry. There is evidence of at least 2 strains of the virus in California. There is degeneration in the phloem region of the bark that may be readily brought out by treating with phloroglucinol. Certain infected plants under glass develop pronounced vein swelling, accompanied by schizogenous cavities, by hypertrophy and hyperplasia in the phloem and bundle sheath and deposits of yellowish material in the cells of phloem ray and bundle sheath. The abscission of portions of the lamina is preceded by early solution of intercellular substance in the narrow abscission zone.

Among the *Prunus* species of particular interest *Prunus avium, P. cerasus, P. demissa*, and *P. persica* seem to be the most susceptible of those studied, while *P. armeniaca, P. communis* and *P. mahaleb* are resistant. Varieties of *P. domestica* have never shown symptoms, but attempts to recover the virus have not been concluded. The plums *P. cerasifera, P. marianna*, and *P. sub-cordata*, appear to be immune or highly resistant.

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LITERATURE CITED

1. HEWITT, W. B. Leaf-scar infection in relation to the olive knot disease. Hilgardia 12: 41–65. 1938.
2. RAWLINS, T. E., and W. T. HORNE. "Buckskin," a destructive graft-infectious disease of the cherry. Phytopath. 21: 331–335. 1931.
3. ————, and K. G. PARKER. Influence of rootstock on susceptibility of sweet cherry to buckskin disease. Phytopath. 24: 1029–1031. 1934.
4. STODDARD, E. M. The "X" disease of peach. Connecticut (New Haven) Agr. Exp. Stat. Circ. 122. 1938.
5. THOMAS, H. EARL, T. E. RAWLINS, and K. G. PARKER. A transmissible leaf-casting yellows of peach. Phytopath. 30: 322–328. 1940.

ARE AMMONIUM SALTS TOXIC TO THE COTTON ROOT ROT FUNGUS?[1]

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INTRODUCTION

Neal, Wester, and Gunn (8) reported little or no growth of the causal organism (*Phymatotrichum omnivorum* (Shear) Duggar) of cotton root rot in a synthetic nutrient solution when the source of nitrogen was NH_4NO_3 or $(NH_4)_2SO_4$. Ezekiel, Taubenhaus, and Fudge (5), using a different synthetic nutrient solution, obtained little or no growth with $(NH_4)_2SO_4$. Growth with this salt, when it occurred in their experiments, was accompanied by a low pH value at the time of harvest. They found NH_4NO_3 was an excellent source of inorganic nitrogen. More recent studies (2, 15) have shown that the type of nutrient solution employed by Neal *et al.* was a poor medium for the best growth response of the root-rot fungus, *P. omnivorum.* Certain aspects of the importance of optimum solutions in estimating the ability of a fungus to utilize nitrogen have been emphasized by Steinberg and Bowling (11). Since the solution employed by Neal *et al.* was unadapted to good growth by *P. omnivorum,* their data on the utilization and toxicity of $(NH_4)_2SO_4$ are of questionable value. The good growth with NH_4NO_3 obtained by Ezekiel *et al.* is associated with the amphoteric physiological nature of this compound. The poor growth reported by them, when $(NH_4)_2SO_4$ was the source of nitrogen, can be attributed to the development of a low pH in the $(NH_4)_2SO_4$ solutions (16).

The use of ammonium compounds has been recommended by Streets (12, 13, 14) for the treatment of perennials infected with root rot. He states, as a result of the apparent recovery of plants treated by heavy applications of $(NH_4)_2SO_4$, that "ammonium sulphate was found to be, in common with other ammonium compounds, toxic to the root-rot fungus when supplied in sufficient concentrations," and "it seems evident that at these high concentrations the immediate effect of ammonium salts is fungicidal or fungistatic rather than nutritional" (13).

Recent studies on the utilization of different forms of inorganic nitrogen by *Phymatotrichum omnivorum* have shown that ammonium compounds are excellent sources of nitrogen in properly constituted and controlled nutrient solutions (16). The ammonium ion was not toxic to the organism, if the development of critical acidity was prevented, in concentrations of 0.0125 or 0.025 M. $(NH_4)_2SO_4$ or equivalent amounts of other ammonium compounds. These results appear to conflict with those of Neal *et al.* and with the conclusions and recommendations of Streets.

This study is intended to furnish some additional information on the nutritional value and toxic effect of various concentrations of ammonium salts in regard to *P. omnivorum.*

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[1] Published with the approval of the Director as Technical Contribution No. 614 of the Texas Agricultural Experiment Station.

MATERIALS AND METHODS

The culture methods employed were an adaptation of those used in earlier studies with this organism concerning the effect of trace elements (2), the nutritional requirements (15), and inorganic nitrogen utilization (16). The inoculum was a standardized agar disc cut from a vigorous Petri-dish culture of isolate No. 53 of *Phymatotrichum omnivorum.* The dry weight of the inoculum disc varied from 3 to 5 mg. Experiments have demonstrated the pathogenicity of this isolate (3), and its response to different nitrogen sources has been investigated (16). The basic nutrient solution contained 0.008 M. K_2HPO_4, 0.003 M. $MgSO_4 \cdot 7H_2O$, 0.002 M. KCl and additions of 2 p.p.m. each of Fe, Mn, and Zn. Glucose was supplied at 40g. per liter. All cultures were incubated at 28° C. The dry weights of the fungal mats were used as a quantitative measure of growth.

The variables in one experiment were the sources of nitrogen, the amount of nitrogen, the presence or absence of 0.0125 m. $CaCO_3$, and the period of incubation. Nitrogen was supplied as $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$ or NH_4NO_3. Seven different levels or concentrations of each of these nitrogenous salts were used. The lowest concentration employed was 0.0063 M., while the highest was 0.075 M. These variations resulted in 21 nutrient solutions differing from each other in the amount of nitrogen or the source of nitrogen. Each of these 21 different solutions was divided into 2 equal portions and $CaCO_3$ was added to one portion in the proper amount to result in a 0.0125 M. concentration when the portion was raised to its final and correct volume. Each type of solution, with and without $CaCO_3$, was prepared in sufficient quantities to yield ten 50-ml. portions, each of which served as a separate culture solution in this experiment. Five replicates of each type were harvested after 20 and 24 days of incubation.

In another experiment soil cultures (Houston black clay) were employed instead of synthetic nutrient solutions. Houston soils are highly colloidal, calcareous, and friable, and are the most common type in the region of Texas where root rot is most prevalent. One hundred g. of air-dry soil was placed in each of several flasks. The moisture content of the soil was determined to be 8 per cent. To one group of these flasks 15 ml. of glucose solution of the proper concentration to furnish 2 g. of glucose per flask were added. To the remaining flasks 15 ml. of water were added. The flasks were plugged, autoclaved, and allowed to cool. Three stock solutions of $(NH_4)_2SO_4$, 0.42, 0.21 and 0.105 M., were prepared and autoclaved. A 5 ml. portion of one or the other of these solutions was added aseptically to each of the various flasks, excepting the check flasks, which received an equal quantity of distilled water. The addition of the 5-ml. portion of one of the ammonium sulphate solutions, or of water, raised the moisture content of each flask to approximately 28 g. per flask. The respective concentrations of supplemental ammonium sulphate in the different flasks might be considered as .075, .0375, .0188 M., and zero. This resulted in the 8 different types of soil culture shown in table 2. All of the flasks were comparable as to amount of soil and moisture. The variables were glucose, present or absent, and the presence of $(NH_4)_2SO_4$ at 3 different levels, or its absence. The flasks were inoculated with discs, plugged, sealed to retard the loss of moisture, and incubated at 28° C.

EXPERIMENTAL RESULTS

The mean dry weights of the fungal mats produced on the different solutions after 20 days' incubation are listed, along with the original pH values (after autoclaving) in table 1. The range of the pH values at the time of harvest also are included. The results after the 24-day period of incubation are omitted, since they are only confirmatory in value. In figure 1 the effects of the source and concentration of the ammonium compounds on the growth of the organism are shown graphically.

The results of the soil cultures after 21 days growth are presented in table 2. No determination of fungal weights was attempted, since the accurate separation of the soil and mycelium was too difficult.

DISCUSSION

The curve for the weights of the fungal mats produced in the different concentrations of NH_4NO_3 without $CaCO_3$ show the usual increase in weights for the first 4 concentrations of this nitrogenous salt (Fig. 1). This is the expected reaction, since it has been shown that the rate of growth is largely dependent upon the amount of nitrogen, if some other factor is not limiting (15). The gradual decrease in the mean weights as the concentration of NH_4NO_3 is increased above 0.0375 M. should not be interpreted as an indication of toxicity. In a preliminary experiment it was found that the heavy mats were produced on the 3 higher concentrations of NH_4NO_3 in 15 days. The weights presented here should be interpreted as representing some autolysis following extremely rapid growth during the early part of the period of incubation. This autolysis is assumed to be connected with an exhaustion of the supply of available carbon (5, 15). The higher pH values in the more concentrated NH_4NO_3 solutions at the time of harvest support the assumption that some autolysis has occurred.

The effect of $CaCO_3$ on growth with the 7 concentrations of NH_4NO_3 varies to some extent with the concentration. The addition of $CaCO_3$ is of little or no value with the lower concentrations, but appears to exert a favorable influence on the higher levels. This favorable effect is more apparent than real. Since $CaCO_3$ tends to delay the rapid growth usually occurring with an excess of NH_4NO_3, the peak is reached at a later date, and less autolysis has occurred at the time of harvest (16). The pH values at the time of harvest agree with this interpretation.

The $(NH_4)_2SO_4$ solutions lacking calcium carbonate were uniformly

TABLE 1.—*The growth responses of P. omnivorum to three different ammonium salts, each employed at seven concentrations, with and without the addition of* $CaCO_3$

Molar conc. of nitrogenous salt	.0125 M. $CaCO_3$	$(NH_4)_2HPO_4$			$(NH_4)_2SO_4$			NH_4NO_3		
		Original pH	At harvest		Original pH	At harvest		Original pH	At harvest	
			pH range	Mean wt.		pH range	Mean wt.		pH range	Mean wt.
				mg.			*mg.*			*mg.*
0.0063	–	6.70	4.6–5.2	251	6.49	2.9–3.0	71	6.43	6.4–6.6	388
	+	6.70	6.0–6.6	206	6.68	5.5–5.6	165	6.34	6.7–7.0	297
0.0125	–	6.79	3.2–3.3	163	6.61	2.9–3.0	65	6.52	5.2–5.9	553
	+	6.71	5.5–6.0	295	6.64	2.9–5.5	281	6.42	6.5–6.8	361
0.0250	–	6.78	3.2–3.3	285	6.58	3.0–3.1	57	6.60	4.8–5.4	566
	+	6.65	4.2–4.9	504	6.62	2.8–3.6	432	6.53	6.8–7.3	593
0.0375	–	6.74	3.1–3.3	372	6.55	3.0–3.1	51	6.47	6.3–6.6	632
	+	6.73	4.3–4.6	540	6.68	2.9–3.9	385	6.47	5.9–6.7	550
0.0500	–	6.70	3.4–5.9	272	6.65	3.1–3.2	52	6.51	6.3–6.7	584
	+	6.74	3.3–3.7	517	6.71	2.8–3.5	403	6.42	5.8–6.1	539
0.0625	–	6.57	3.7–6.3	234	6.68	3.1–3.5	51	6.42	5.7–6.8	510
	+	6.53	4.0–4.5	606	6.68	2.9–4.1	392	6.38	6.0–6.3	546
0.0750	–	6.55	6.2–6.4	28	6.68	3.1–3.4	50	6.38	6.8–7.3	391
	+	6.52	4.5–5.9	530	6.68	3.4–4.9	351	6.39	5.7–6.2	472

unsatisfactory for good growth of this fungus. The explanation is apparent in most of these cases from the pH values at the time of harvest (Table 1). In all of these solutions, regardless of the concentration of $(NH_4)_2SO_4$, the pH was found to be between 2.9 and 3.5. The addition of $CaCO_3$ tends to retard the development of critically low pH values in the ammonium sulphate solutions, thus permitting relatively good growth in all solutions where nitrogen is not a limiting factor. The data indicate that the failure of the fungus to produce good growth with ammonium sulphate is not the result of any direct toxic action of the ammonium ion, or of ammonium sulphate, but it is connected with the development of acidity in the solutions as growth progresses. Calcium carbonate delays this acid development. There is an additional effect of the Ca ion on the utilization of ammonium when the sulphate content of the nutrient solution is appreciably increased (16).

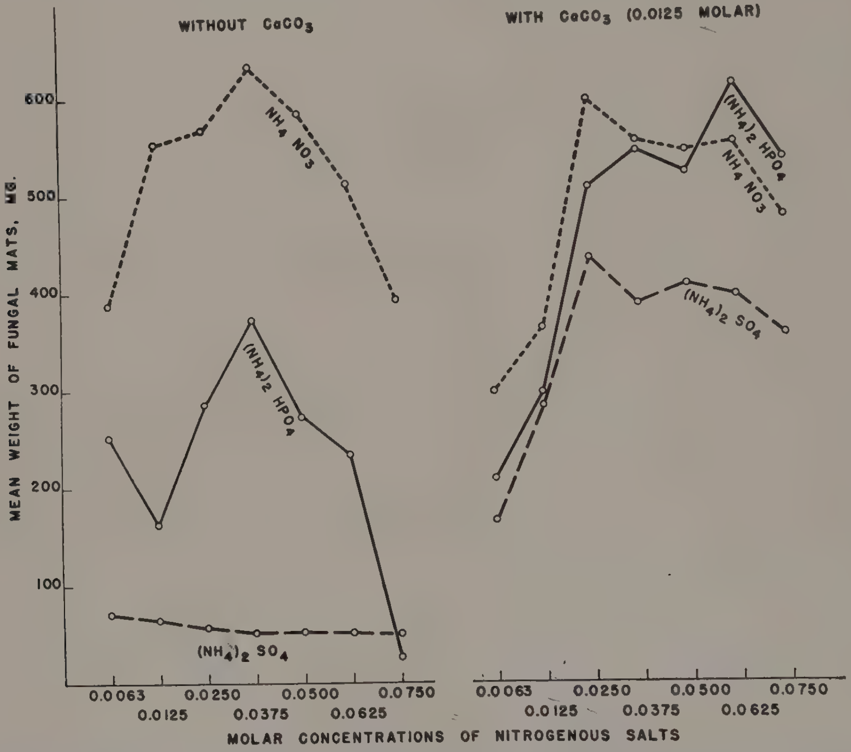

FIG. 1. Curves representing the mean growth responses of *Phymatotrichum omnivorum* to different concentrations of ammonium salts, with and without addition of $CaCO_3$.

The results obtained from the ammonium phosphate solutions, with and without calcium carbonate, furnish additional data on the nutritive value of ammonium (Table 1 and Fig. 1). When nitrogen ceases to be limiting, and the pH values do not decrease too much, fair to good growth occurs with most of the ammonium phosphate solutions, even when calcium carbonate is lacking. Each increase in the amount of ammonium phosphate raises the buffering capacity of the solution and, simultaneously, tends to increase the rate and amount of growth by increasing the supply of nitrogen. Heavier mats are to be expected, therefore, as the content of this salt is increased within limits. The low solubilities of the phosphates of the essential trace elements and the increased concentration of the phosphate radical might account for the poor growth in the 3 more concentrated solutions. However, when calcium carbonate was added to the different concentrations of ammonium phosphate all of the solutions, including the higher concentrations of this salt, produced heavy fungal mats.

The results obtained from the use of the several concentrations of ammonium sulphate and ammonium phosphate in synthetic nutrient solutions, with and without calcium carbonate, do not present any basis for assuming that the ammonium ion, *per se,* is toxic to *P. omnivorum.* If poor growth is obtained with the ammonium salts, it apparently can be attributed either to the rapid development of critical acidity or to a reduction in the availability of the trace elements (2). When the composition and properties of the solution are suitably modified by the addition of $CaCO_3$ no evidence of toxicity can be found, even in concentrations as high as 0.075 M. $(NH_4)_2SO_4$ or $(NH_4)_2HPO_4$.

The results obtained from the soil-culture experiment agree with those obtained from the nutrient solutions in regard to the toxicity and nutrient value of ammonium compounds for the growth of the fungus (Table 2). The addition of $(NH_4)_2SO_4$ to the soil favors rapid and abundant growth of the fungus if the available carbon is increased by the addition of glucose. The rapid growth of the fungus and the production of sclerotia under these conditions indicate that the fungus, when growing saprophytically in the soil, is favored by the addition of ammonium sulphate, if some other factor is not limiting. Since these soils are highly calcareous and have an abundant alkaline reserve the utilization of ammonium nitrogen does not produce any noticeably unfavorable reaction in the soil, as indicated by fungal growth. The response occurring in soil cultures is similar to that in the synthetic nutrient solutions containing $CaCO_3$.

In the soil cultures receiving no additional carbon no harmful or toxic effect of high concentrations of ammonium is observed. It is clear that nitrogen is not the limiting factor in this case, since the addition of extra amounts of ammonium sulphate is without appreciable effects. The lack of any fungicidal action, when carbon is limiting, is as significant as the beneficial effects of ammonium sulphate on mycelial development and sclerotial formation when carbon ceases to be limiting.

The higher concentrations of ammonium sulphate and ammonium phosphate used in these experiments contain ammonium in amounts equal to, or greater than, that recommended by Streets (12, 13) for the treatment of plants attacked by *Phymatotrichum omnivorum.* One of the recommended methods of treatment involves the use of a solution containing 1 lb. of com-

TABLE 2.—*The growth of Phymatotrichum omnivorum in soil as influenced by available carbon and the addition of ammonium sulphate*

Soil supplement		Amount of growth	Type of growth		
Glucose	$(NH_4)_2SO_4$		Surface mycelium	Strand development	Sclerotial production
	M. conc.				
2	.0750	Abundant	Abundant	Good	Fair
2	.0375	"	"	"	Good
2	.0188	"	"	"	Abundant
2	.0000	Fair	None	Fair	Scant
0	.0750	Scant	"	Scant	None
0	.0375	"	"	"	"
0	.0188	"	"	"	"
0	.0000	"	"	"	"

mercial ammonium sulphate dissolved in 10 gal. of water. If one assumes this ammonium sulphate is pure, the resulting solution would be approximately 0.075 M., which was the highest concentration used in the experiments reported in tables 1 and 2. This solution is added to the soil at the rate of 1 gal. per sq. ft. and is followed by heavy watering to carry the solution into the soil to a depth of 3 or 4 feet. After watering, the concentration of ammonium sulphate in the soil would be lowered into the range shown to be very satisfactory for the growth of the fungus, when other factors, such as the pH, are not limiting (16). The root rot of cotton and other plants is most prevalent and serious in the calcareous and alkaline soils of the Southwest. In such soils it is difficult to assume that this recommended treatment for diseased plants would produce a pH throughout the soil that would be low enough to retard the growth of or destroy the organism.

Free ammonia has been shown toxic to the vegetative hyphae and sclerotia of the root-rot organism (8). There is the possibility in the case of alkaline soils that some free ammonia might be liberated in the soil following the application of the solutions of ammonium salts. The amount of liberated ammonia, if any, would be insufficient to be toxic to the fungus in the soil, since it has been shown that free ammonia is rapidly fixed in a relatively stable and non-toxic condition in heavy colloidal systems (17). One seems justified in ruling out the possibility and probability of any toxicity that can be attributed to the liberation of free ammonia under ordinary field conditions in alkaline soils.

Another possibility deserves consideration. According to Mevius and Engle's interpretation of the toxic action of ammonium compounds (7), free ammonia might accumulate in the tissue of the host. Thus a toxic condition might develop in the host tissue or in the fungus that would destroy the organism parasitizing the plant. Nightingale (10) doubts this interpretation, since there is such a pronounced tendency for living cells to maintain a relatively constant internal pH value under varying external conditions. This tendency would preclude the probability of the accumulation of toxic amounts of free ammonia in the host tissue by the differential absorption of ammonium. The data in tables 1 and 2 indicate that ammonia does not accumulate in the fungus in toxic quantities when it is growing in media containing high concentrations of ammonium salts.

The apparent fungicidal action of ammonium salts towards *Phymatotrichum omnivorum* in certain soils might be attributed to another effect without assuming either the liberation of significant amounts of free ammonia or the direct toxicity of the ammonium ion. The pH and composition of the soil solution immediately after the application of the concentrated ammonium solutions would be favorable for the rapid absorption of the ammonium ion by the roots of the host. A rapid absorption of ammonium from certain soils by the fungus and by the host plant might produce an acid condition at their surface and in the soil immediately adjacent to them, if the evidence from Nightingale's culture studies (9), and those of Davidson and Shive (4), can be considered applicable in this connection. Such an indirect effect of ammonium sulphate or phosphate would be comparable to the more direct action of sulphuric acid, which has been used with some success in checking root rot in soil (13). This effect, however, would be localized in small portions of the soil and would be extremely temporary in the highly alkaline and calcareous types of soils in which root rot is most prevalent. It is, therefore, doubtful if acidity resulting from the rapid absorption of ammonium would form a basis for the control of root rot in nature by the heavy application of ammonium salts.

In fact, there seems to be no logical basis for assuming that ammonium salts are toxic to the fungus or that the treatment of infected plants with ammonium salts will produce any important fungicidal or fungistatic compounds in the soil.

The favorable action of high nitrogen fertilizers in decreasing the loss from root rot, which has been reported by Jordan, Nelson, and Adams, furnishes additional information in regard to the possible action of ammonium compounds (6). They report the heavy applications of a mixture of nitrate and ammonium fertilizers decrease the percentage of dead cotton plants in root-rot-infested Wilson soils. Adams *et al.* have reported a significant negative correlation between the nitrogen content of the roots, especially the bark, and the percentage mortality from the disease (1). They suggest the possibility that variations in the chemical composition of the host tissue might account for increased resistance to the fungus.

A possible explanation of the apparent recovery of some plants following the heavy application of nitrogenous fertilizers, such as ammonium salts, would be the physiological response of the host plants. If the carbohydrate content of the older portions of the roots did not become limiting following the heavy application of readily available nitrogen, there would be a rapid synthesis of organic nitrogenous compounds in the root and an accompanying acceleration of the rate of development of new lateral roots. A rapid development of new lateral roots might permit the host plant to tolerate, temporarily, at least, some parasitism without the appearance of aboveground symptoms of infection. The favorable action of the nitrogenous fertilizers on root development accompanying the recovery of pecan trees has been illustrated by Streets (14). According to this view the favorable effects of ammonium fertilizers on plants already attacked by the fungus and the beneficial effects resulting from the use of large quantities of nitrogenous fertilizers on heavily infested soils are not to be attributed to any direct toxic action on the fungus but are dependent upon the favorable responses of the root system of the host plant.

CONCLUSIONS AND SUMMARY

The results from nutrient solutions using several concentrations of ammonium sulphate and ammonium phosphate, with and without the addition of $CaCO_3$, do not present any basis for assuming that the ammonium ion, *per se,* is toxic to *Phymatotrichum omnivorum.* An acid condition, which inhibits further growth, rapidly develops when *P. omnivorum* is cultured with ammonium salts as the source of nitrogen, if the nutrient solution is not properly constituted. The addition of $CaCO_3$ is effective in preventing the development of this critically acid condition.

The results of soil-culture studies show that ammonium nitrogen is a good source of nitrogen for the fungus. No evidence of toxicity was observed.

The beneficial effects of the treatment of diseased plants with ammonium compounds are thought to be the result of the growth responses of the host plant to the additional amount of available nitrogen.

In view of the lack of any indication of toxicity of the ammonium ion, it seems doubtful that complete eradication resulting in permanent control of the root rot can be obtained by the use of ammonium salts.

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LITERATURE CITED

1. ADAMS, J. E., R. C. WILSON, L. E. HESSLER, AND D. R. ERGLE. Chemistry and growth of cotton in relation to soil fertility and root rot. Proc. Soil. Science Soc. Amer. **4**: 329–332. 1939.
2. BLANK, LESTER M. Response of *Phymatotrichum omnivorum* to certain trace elements. Jour. Agr. Res. [U. S.] **62**: 129–159. 1941.
3. ————. The susceptibility of cotton seedlings to *Phymatotrichum omnivorum.* Phytopath. **30**: 1033–1041. 1940.
4. DAVIDSON, O. W., AND J. W. SHIVE. The influence of the Hydrogen-ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand cultures. Soil Sci. **37**: 357–385. 1934.
5. EZEKIEL, WALTER N., J. J. TAUBENHAUS, AND J. F. FUDGE. Nutritional requirements of the root-rot fungus, *Phymatotrichum omnivorum.* Plant Physiol. **9**: 187–216. 1934.
6. JORDAN, H. V., H. A. NELSON, AND J. E. ADAMS. Relation of fertilizers, crop residues, and tillage to yields of cotton and incidence of root rot. Proc. Soil Science Soc. Amer. **4**: 325–328. 1939.

7. MEVIUS, W., AND H. ENGLE. Die Wirkung der Ammoniumsalze in ihrer Abhängigkeit von der Wasserstoffionenkonzentration. Planta 9: 1–83. 1929.
8. NEAL, DAVID C., R. E. WESTER, AND KENNETH C. GUNN. Growth of the cotton root-rot fungus in synthetic media, and the toxic effect of ammonia on the fungus. Jour. Agr. Res. [U. S.] **47**: 107–118. 1933.
9. NIGHTINGALE, GORDON T. Ammonium and nitrate nutrition of dormant Delicious apple trees at 48° F. Bot. Gaz. **95**: 437–452. 1934.
10. ————. The nitrogen nutrition of green plants. Bot. Rev. **3**: 85–174. 1937.
11. STEINBERG, ROBERT A., AND JOHN D. BOWLING. Optimum solutions as physiological reference standards in estimating nitrogen utilization by *Aspergillus niger*. Jour. Agr. Res. [U. S.] **58**: 717–732. 1939.
12. STREETS, R. B. The treatment of deciduous fruit trees and nut trees infected by *Phymatotrichum omnivorum* with ammonium compounds. Science (n.s.) **79**: 417–418. 1934.
13. ————. Phymatotrichum (cotton or Texas) root rot in Arizona. Ariz. Agr. Exp. Stat. Tech. Bull. **71**: 299–410. 1937.
14. ————. Phymatotrichum (Texas or cotton) root rot in Arizona. Fiftieth Ann. Rpt. Ariz. Agr. Expt. Stat. (1938): 89–91. 1939.
15. TALLEY, PAUL J., AND LESTER M. BLANK. A critical study of the nutritional requirements of *Phymatotrichum omnivorum*. Plant Physiol. **16**: 1–18. 1941.
16. ————, AND ————. Some factors influencing the utilization of inorganic nitrogen by the root rot fungus. Plant Physiol. **16**: 1–18. 1941.
17. WLLIS, L. G., AND J. R. PILAND. Ammonium calcium balance: a concentrated fertilizer problem. Soil Sci. **31**: 5–23. 1931.

A XYLARIA TUBER ROT OF POTATO

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INTRODUCTION

A field rot caused by a species of *Xylaria* occurs on potato tubers grown during the winter months in the calcareous marl soils of southern Florida. The rot was first observed by potato inspectors on freshly washed potatoes in packing houses at Goulds, Florida, during the 1936 shipping season, and a limited number of specimens have been collected annually since then. It was first mistaken for the somewhat similar decay of potato tubers caused by *Armillaria mellea* Vahl. which was described by Bailey[1] in 1914 as occurring in the Pacific Northwest. Its presence in other potato sections in Florida has not been ascertained.

The causal organism was isolated from affected tubers in 1937 and was grown on several kinds of culture media, where its behavior was suggestive of *Xylaria*. Positive identification from the cultures was impossible because of failure of the isolates to produce ascospores. In April, 1940, fructifications of a *Xylaria* were discovered arising from decaying stumps and branches of a common species of willow in a potato field near Homestead, Florida. Isolation cultures from ascospores were identical with the original isolates obtained from potato tubers. Development of the typical field rot on potato tubers in close proximity to willow wood infected by the *Xylaria* was presumptive evidence that the latter was the causal agent for the potato rot.

SYMPTOMS

The most distinctive characteristic of Xylaria rot is the presence of black rhizomorphs of the fungus which become firmly attached to the surface of the tubers (Fig. 1, B and C). These rhizomorphs may be simple or variously branched and range from threadlike to 3 mm. in thickness. The points of attachment to the tubers become centers of infection, which results in the production of a dry rot.

The decayed areas are circular, sunken, and typically light-tan at the centers, shading to light-brown at the margins. The skin of the tuber may remain more or less intact and held in place by a plate of mycelium, which usually forms just beneath the surface, but the deeper-lying tissues generally are completely destroyed and replaced by fungal tissue as the invasion slowly advances. The line of demarcation between healthy and invaded tissue usually is quite sharp (Fig. 1, A). Secondary invasion of the decayed areas of soft-rot bacteria, soil fungi, and insects or mites frequently occurs and hastens the disintegration of the tubers.

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[1] Bailey, F. D. Notes on potato diseases from the Northwest. Phytopath. 4: 321–322. 1914.

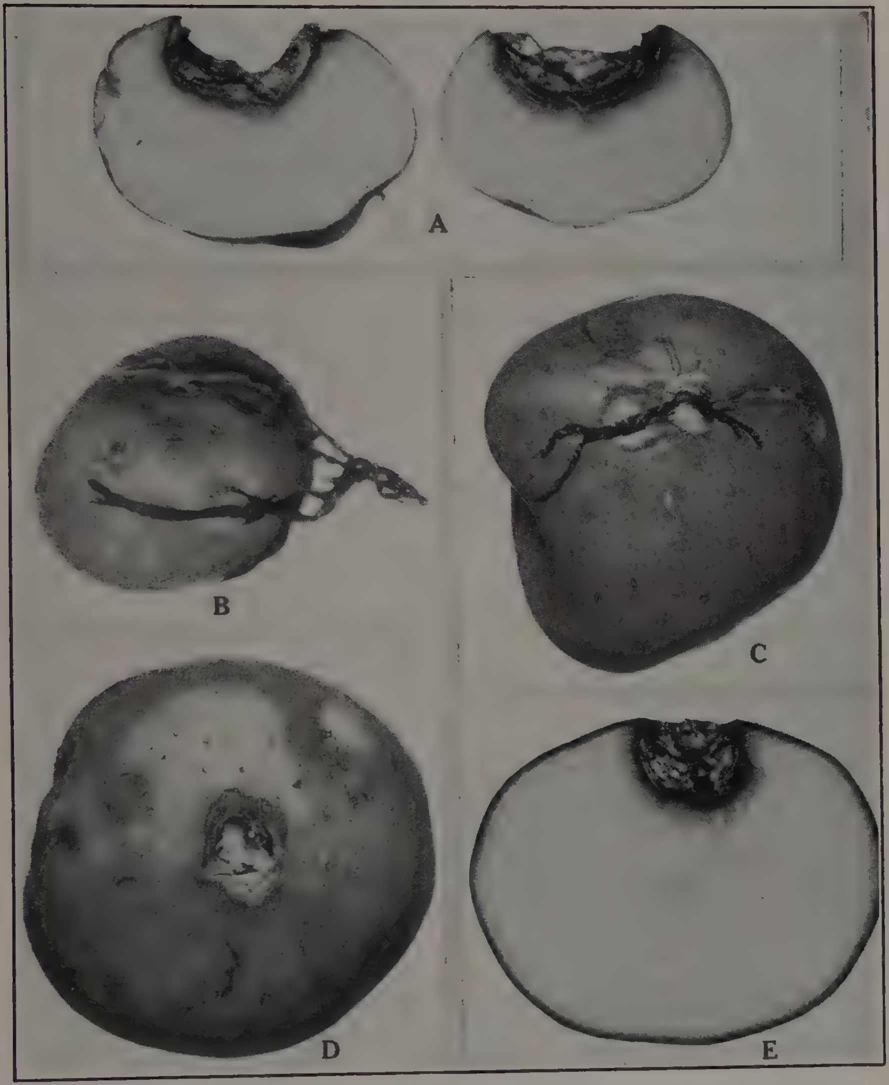

FIG. 1. Xylaria rot of Bliss Triumph potatoes. A. Cross section of tubers decayed by *Xylaria*. B. Rhizomorphs of the fungus attached to a tuber. C. Dry rot developing at several points of attachment. D. Dry rot of tuber thirty days after inoculation from pure culture of *X. apiculata*. E. Cross section of tuber in D.

INCIDENCE OF XYLARIA ROT

The rot is of minor importance, but is of considerable interest because there appears to be no previous report of a species of *Xylaria* parasitizing the potato. Most of the observed specimens were from farms where the land had been recently cleared and contained numerous willow roots and stumps. Even on farms where the *Xylaria* was rather abundant, the loss from infection of potato tubers was of little consequence. The rot was absent, or nearly so, on land that had been long farmed annually.

PATHOGENICITY

Pathogenicity was proved by artificial inoculations. After sterilizing the surface of potato tubers, the skin was broken by lightly scratching the surface with the point of a flamed scalpel. A young culture of the fungus with some of the medium adhering was lifted from a potato dextrose-agar plate, placed in contact with the wounded area, and the inoculated tubers were incubated at room temperatures in culture chambers. A week later the fungus was well established in the potato tissues (Fig. 1, D), but invasion proceeded at a very slow rate in no case exceeding 15 mm. by the end of 30 days. The character of the rot was similar to that produced by natural infections, except that more fungous hyphae were present in the decay cavities (Fig. 1, E). It is probable that the small insects and mites generally present in the decay spots that develop in the soil feed upon the fungus mycelium.

MORPHOLOGY AND IDENTITY OF THE FUNGUS

Stromata arising from willow wood show considerable variation in size and conformation. These structures are upright, solitary to several cespi-

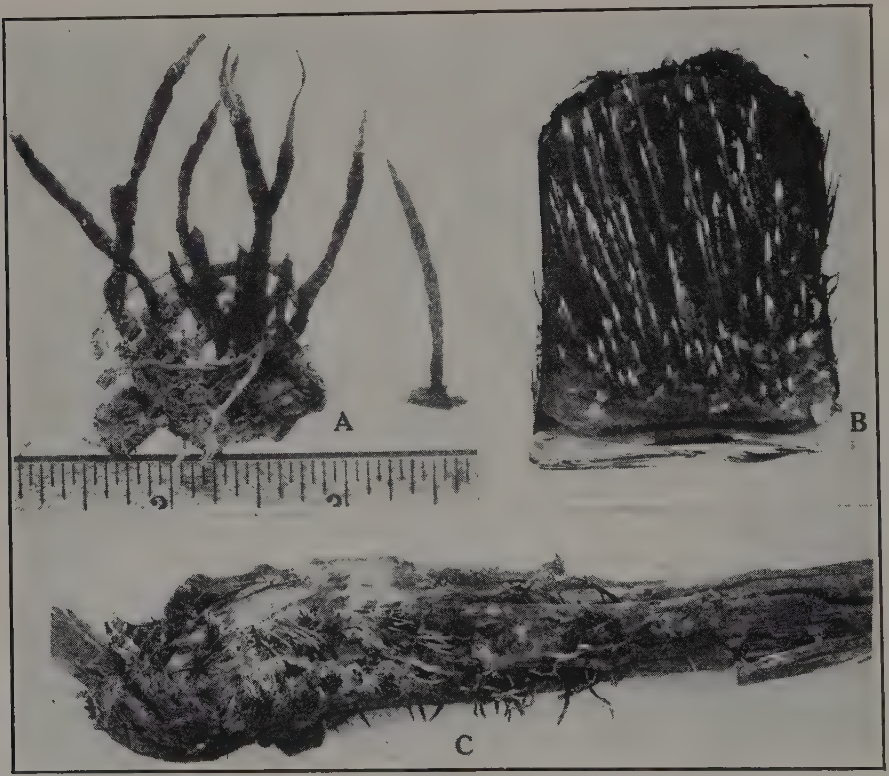

FIG. 2. *Xylaria apiculata.* A. Mature stromata from willow wood. B. Culture 18 days old on potato-dextrose agar of original isolate from potato tuber, showing abundant production of sterile stromata. C. Immature stromata arising from willow wood.

tose, and are simple or occasionally branched (Fig. 2, A and C). They vary in length from .5 to 8 cm. or more and from .5 to 3 mm. in width. Stipes range from obsolete to several cm. long.

Immature stromata exhibit strong positive phototropism, which accounts for considerable curvature of the clubs (Fig. 2, C) if the direction of light is variable. They are typically cream- to tan-color at the apiculate tips, shading to olivaceous-brown and then to black below, where the surface is densely villose from the numerous dark hyphae arising from the surface. The surface just below the tips sometimes becomes powdery from the copious production of sub-hyaline to yellowish, continuous, fusiform-elliptical conidia, 6–7 × 2–3 μ in size. As the stromata approach maturity, the surface of the fertile portion loses its villose character. It remains roughened for a time from the adhering fragments of hyphae but finally, when full maturity is reached, becomes somewhat undulate and prominently striate to resemble the fissured bark of a tree (Fig. 2, A). The asci are 8-spored, cylindrical, hyaline, and stipitate, 135–170 × 8–11 μ. The ascospores are uniseriate, inequilaterally elliptical, rounded at the ends, brown, continuous or sometimes with an indistinct septum, 16–25 × 4.5–7 μ (average 19.5 × 6 μ).

Growth is luxuriant on potato-dextrose agar. Immature stromata are produced in large numbers on this medium, where their appearance is similar to those produced on natural substrata (Fig. 2, B). Conidia are frequently produced near the tips of these structures, but ascospores have not been produced thus far on laboratory media.

The apiculate stromatal tips, ascospore measurements, and characteristic markings on the surface of the spore-bearing areas, agree closely with Lloyd's[2] descriptions and illustrations of *Xylaria apiculata* Cooke. There is little doubt that the potato-rotting *Xylaria* is *X. apiculata* if Lloyd's interpretation of this species is correct. According to Erdman West,[3] who identified the fungus for the writer, the same species has been collected on rotten wood from various places in Florida.

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[2] Lloyd, C. G. Notes on Xylarias. Mycological Notes 5: 20–21 and 675–679. 1916–1919.

[3] Mycologist, Florida Agricultural Experiment Stations, Gainesville, Florida.

NEW FACTS ABOUT EASTERN SNOWMOLD

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The term "snowmold" was applied by Dahl (2) to a disease of fine turf caused by *Fusarium nivale.* Snowmold is noticeable as soon as the snow melts and is characterized by dead areas irregularly circular in outline, whitish gray in appearance. The leaves have a bleached appearance and feel slimy when wet. Well-developed patches of dead turf often exhibit a pinkish cast when exposed to sunlight. Davis (3) reported snowmold and brown patch to be caused by *Sclerotium rhizoides.* Broadfoot (1) was able to produce disease on Chewings fescue with a species of *Fusarium, Rhizoctonia,* and unidentified basidiomycete which he had isolated from grass suffering from snowmold. Recently, Remsberg (7) has applied the name "snowmold" to a disease caused by *Typhula itoana,* a sclerotial basidiomycete that is becoming increasingly important on turf and lawn grasses in the eastern United States. Dahl makes no mention of sclerotial fungi in his paper (2), but, in an earlier publication with Monteith (4), the following statement appears: "Occasionally patches of snowmold are found in which the leaves of the injured grass have small black or brown bodies (sclerotia) scattered over them. These are resting bodies of fungi which, although associated with the snowmold injury, have not been proved to be the causal organisms."

What seemed to be snowmold first came to the writer's attention in March, 1939, when a severe outbreak occurred on the College organic fertilizer plots, which were seeded to Colonial bent. Monteith and Dahl (4), Dahl (2), and Pirone (5) list Colonial bent as a resistant species. To find it so severely diseased merited closer investigation. None of the dead areas showed a pinkish cast in sunlight, nor did the dead grass feel slimy when wet. Individual grass blades and culms showed numerous reddish brown sclerotia embedded in the tissues. Isolations from this material at both room temperature and 13° C. yielded only cultures of a sclerotia-producing basidiomycete. In order to check the technique employed, snowmolded turf was secured from Wayzata, Minnesota, through the kindness of L. J. Feser, and parallel isolations were made. A *Fusarium* was readily isolated from the Minnesota turf, although an occasional grass plant yielded a sclerotial basidiomycete quite unlike the one isolated from local turf. Specimens of snowmold collected from other districts in Pennsylvania failed to yield anything but isolates of a sclerotial basidiomycete. Cultures of this local organism were identified by Miss Remsberg as *Typhula itoana* Imai (6). The sclerotial isolate from Minnesota turf was believed by her to be an undescribed species of *Typhula.*

A broadening of the established concept of "snowmold" has been necessary in order that the disease caused by *Typhula* spp. may be included.

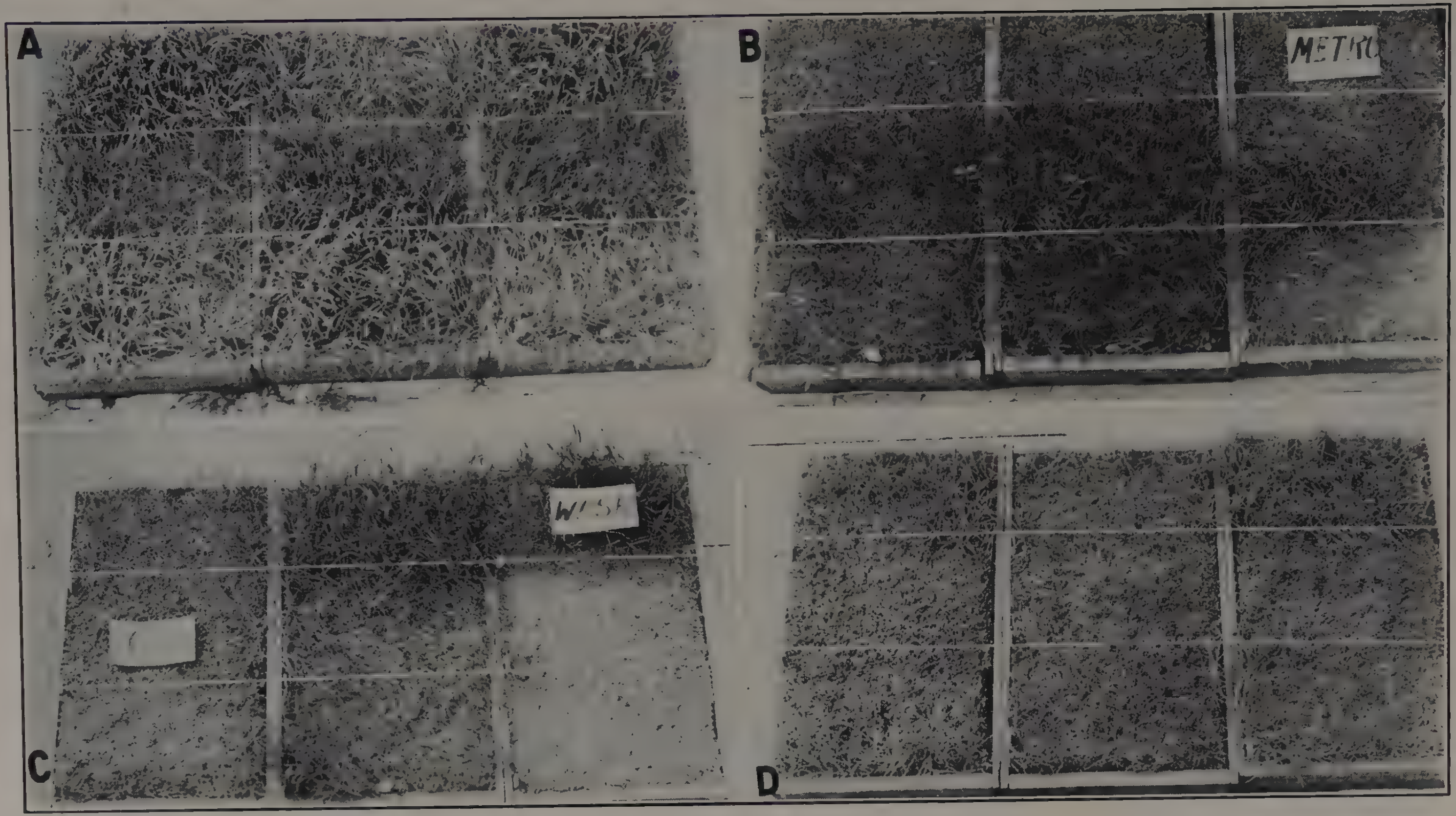

FIG. 1. Reaction of Fine turf grasses to artificial inoculation of *Typhula itoana*. Three replicates per group. Grasses listed from top to bottom. A. 6 (29) 1; 6 (29) 4; and 2 (29) 4. B. Metropolitan; 6 (29) 6; and 5 (29) 1. C. Washington; C_1 (Monteith); and 3 (29) 6. D. 3 (29) 3; 1 (31) 2; and 7 (29) 3. (Plants inoculated Aug. 7, 1940; photographed and data taken Jan. 3, 1941.)

The concept of disease resistance must also be broadened to include the reaction of standard varieties to this pathogen. With this purpose in mind the writer undertook to test for resistance to snowmold several strains and selections of fine turf that have been under agronomic observation for several years. The results are given in table 1 and figure 1.

TABLE 1.—*Reaction of several strains of Agrostis palustris to artificial inoculation by the eastern snowmold organism, Typhula itoana, compared with reaction to Fusarium nivale as published*[a]

Variety	Origin	Percentage recovery from snowmold				Reaction to	
		Replicate			Ave. of Replicates	Typhula	Fusarium
		1	2	3			
Metropolitan		5	5	5	5	V S	R
Washington		25	80	40	48	M S	M S
2 (29) 4	South	80	80	100	86	R	
3 (29) 3	German	40	30	60	43	M S	
3 (29) 6		10	15	5	10	V S	
5 (29) 1	Mixed	25	50	30	35	S	
1 (31) 2	Bent	5	5	5	5	V S	
6 (29) 1	Washington	100	90	70	86	R	
6 (29) 4	"	80	90	90	86	R	
6 (20) 6	"	50	100	40	63	M S	
7 (29) 3	"	20	30	35	28	S	
C$_1$ (Monteith)[b]	?	5	10	5	6	V S	
Astoria	(*Agrostis tenuis*) Colonial	Matural infection in field				S	R

R = resistant; M S = moderately susceptible; S = susceptible; V S = very susceptible.

[a] Refrigeration equipment of U. S. Regional Pasture Research Laboratory was used in this study.

[b] John Monteith reports C$_1$ to be resistant to large brown patch.

These data dictate a new classification of the disease resistance of fine turf, based on the causal organism present. The work of Remsberg (7) and the writer indicate that snowmold in the eastern part of United States is caused by *Typhula itoana* rather than *Fusarium nivale.* Although there is at present not a single item of evidence to cast doubt on the efficacy of the standard chemical control recommendations for greens, such means of control are impractical for snowmold on fairway and pasture grasses. In any grass-breeding program the known disease resistance of parental material is invaluable; any such program in the northern tier of States must take snowmold into account. It does seem imperative, in the light of the evidence presented above, that agronomists and pathologists reclassify their grasses with respect to the eastern snowmold caused by *Typhula itoana.*

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LITERATURE CITED

1. BROADFOOT, W. C. Experiments on the chemical control of snowmould of turf in Alberta. Scientific Agriculture **16**: 615–618. 1936.
2. DAHL, ARNOLD S. Snowmold of turf grasses as caused by *Fusarium nivale.* Phytopath. **24**: 197–214. 1934.
3. DAVIS, W. H. Snowmold and brown patch caused by *Sclerotium rhizoides.* Phytopath. **23**: 8. 1933.
4. MONTIETH, JOHN, JR., AND ARNOLD S. DAHL. Turf diseases and their control. U. S. Golf Assocn. Greens Sect. Bull. **12**: 140–144. 1932.
5. PIRONE, P. P. Turf diseases and their control. In New Jersey Agr. Exp. Stat. "Nursery Disease Notes" **10**: 23–26. 1937.
6. REMSBERG, RUTH E. Studies in the genus *Typhula.* Mycologia **32**: 52–96. 1940.
7. ———. The snow molds of grains and grasses caused by *Typhula itoana* and *Typhula idahoensis.* Phytopath. **30**: 178–180. 1940.

SCLEROTIUM ROLFSII ON COTTON IN ARIZONA

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Sclerotium rolfsii Saccardo, the cause of southern sclerotial rot on many hosts,[1] extensively attacked the cotton crop in parts of the Salt River Valley of Arizona, the past season, and has appeared also on cotton in the Safford district and in Pinal County. Worst affected was an area in the former location, embracing about 600 acres, in which many plants were killed. Presence of a disease in the district was reported to the senior writer who discovered the infection. Cultures thereafter made from the diseased plants gave unmistakable mycelia and sclerotia of *S. rolfsii.*

The soil of that district worst affected by southern sclerotial rot is infested with the common nematode. The resulting root knot has reduced the yield in past seasons, but has never killed many plants. Death of the plants was first reported to the writers in July. Subsequent field inspection revealed two sets of signs and symptoms of disease. Many plants were dwarfed, a condition regularly found in cotton fields infested with the common nematode that was attributed to root knot. The diagnosis of root knot, based upon the appearance of the aerial parts of cotton plants, was verified by the finding of knots on the roots of the dwarfed individuals (Fig. 1, *E*, *c*). A second indication of the presence of disease was the occurrence of extensive gaps (Fig. 1, *D*) in the rows; there were also dead (Fig. 1, *C*), brown plants and wilting plants (Fig. 1, *B*). On the stem bases and upper roots (Fig. 1, *E*, *a*) of diseased, dying and dead plants, mycelial mats and sclerotia were found that proved, on laboratory examination, to be structures of *Sclerotium rolfsii.* Mycelia also occurred on the crowns of apparently healthy plants (Fig. 1, *A*) in which the disease was incipient. A further symptom, not heretofore mentioned in literature, was a swelling on the main stem of many affected plants in the region of the soil line. That the appearance is a true swelling and not an illusory enlargement, mistakenly attributed[2] to constriction of the stem, may be seen by a glance at figure 1, *E*, *b*. The etiology of the swelling has not yet been worked out. It has, however, been observed that the enlargement usually occurs on the stunted, very unthrifty plants that soon wilt under slight water stress. Although the fungus, under Arizona conditions, seems capable of infecting cotton plants at any stage in their growth, the enlargement of the stem apparently is caused by seedling infection.

Surveys of the various cotton districts in the Salt River Valley and Pinal County disclosed a very light but definite infection over the entire area. Areas that had borne no other crop but cotton showed a few dying plants.

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[1] Weber, George F. Blight of carrots caused by *Sclerotium rolfsii* with geographic distribution and host range of the fungus. Phytopath. 21: 1129–1140. 1931.

[2] Ezekiel, Walter N., and J. J. Taubenhaus. A disease of young cotton plants caused by *Sclerotium rolfsii.* Phytopath. 21: 1191–1194. 1931.

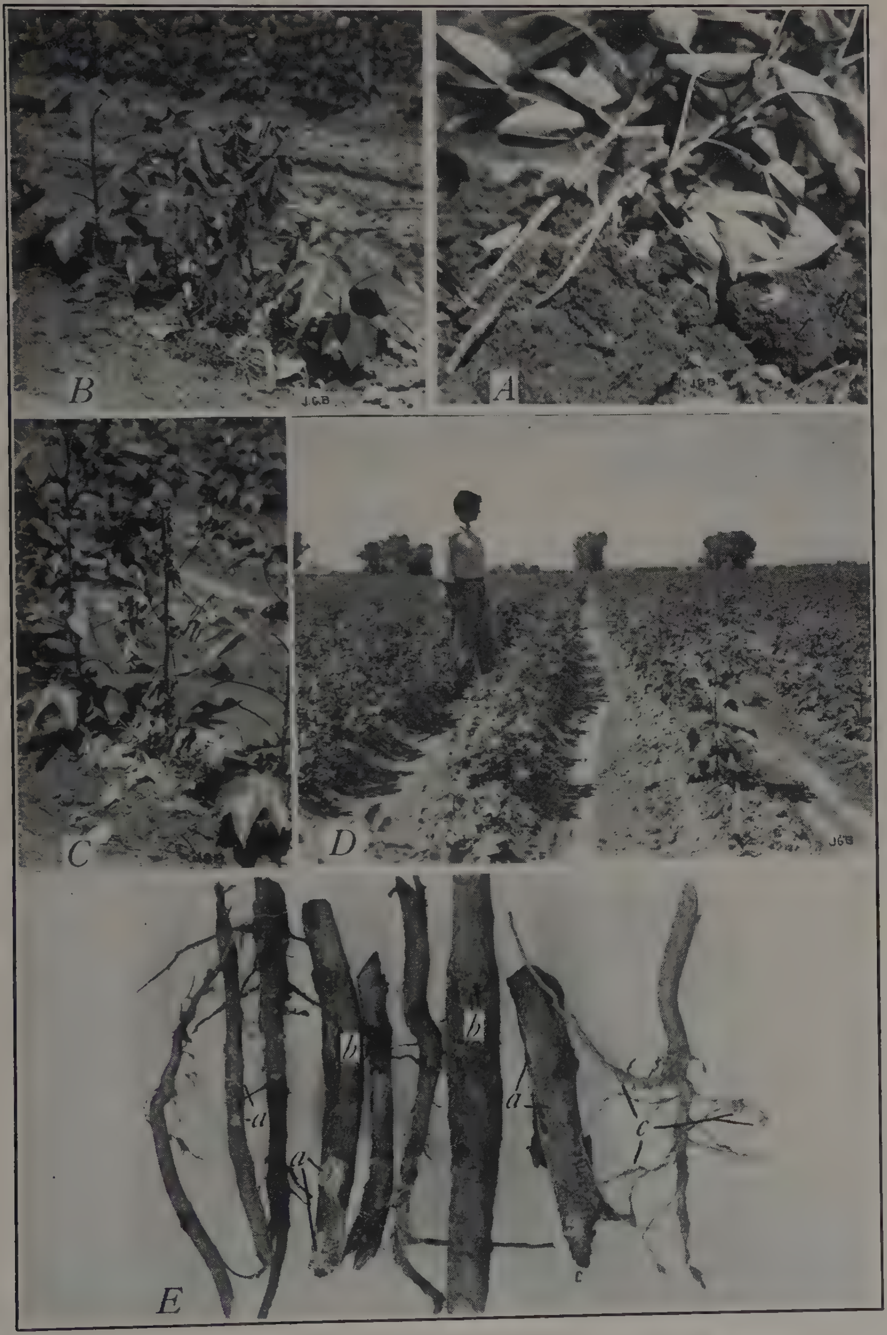

FIG. 1. *A*. Mycelium of *Sclerotium rolfsii* on the crown of an apparently healthy plant. *B*. Acala cotton plant wilting in the field as a result of an attack by *Sclerotium rolfsii*. *C*. Dead, brown plant of Acala cotton killed by southern sclerotial rot. *D*. Acala cotton, right, and S × P cotton, left, affected by southern sclerotial rot. Gaps in the rows caused by the disease. The field of S × P cotton later became as badly damaged as the Acala variety. *E*. Pieces of stems and roots of cotton plants attacked by *S. rolfsii*. The fourth, fifth and eighth pieces from the left were dug up from the soil, having been plowed under from the preceding crop: *a*, the fungus; *b*, swelling at crown; *c*, nematode galls.

By far the most significant fact was the discovery that many fields that showed no dying plants had a very large amount of inoculum present on the decaying stalks of the preceding cotton crop (Fig. 1, *E*, *a*) that had been plowed under.

Numerous cotton fields, free from the root-knot nematode, showed considerable *Sclerotium rolfsii,* which continued to spread in rather sporadic intervals following irrigations and summer rains throughout the growing season. Examination of these infested fields about December 16, one month after a killing frost, revealed much activity of the fungus, as evidenced by mycelial fans and many newly formed sclerotia.

The original source of the infestation of cotton fields with *Sclerotium rolfsii* in the Salt River Valley is not clear. The fungus was first observed[3] in Arizona in 1936 on larkspurs. Later, it appeared on sugar beets on a ranch about 150 miles distant from the site of the first outbreak. The next infestation occurred in beet fields in the Salt River Valley some 15 miles east of the cotton district mentioned in this paper. There were no beet fields near the cotton fields worst affected during the past season and the entire area has had no beet history whatsoever. Local infection of the cotton plants appears to have been made more certain and continuous by the extensive growth of the fungus on decaying cotton stalks in the soil that were plowed under from last year's crop. Pieces of the stalks were abundantly matted with mycelial fans of *Sclerotium rolfsii* and usually also showed many sclerotia.

Southern sclerotial rot in Arizona fields was worse, apparently, on mature cotton plants than has heretofore been observed. Ezekiel and Taubenhaus[2] found one mature plant that appeared to be dying from the effects of the rot, but their attempts to inoculate such plants with *Sclerotium rolfsii* failed.

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[3] Brown, J. G. Southern sclerotial rot. Arizona Agr. Exp. Stat. Ann. Rept. 49: 70. 1938.

PHYTOPATHOLOGICAL NOTES

Poinsettia Scab Caused by Sphaceloma.—Early in July, 1940, the writer's attention was called to an unusual disease of poinsettia (*Euphorbia pulcherrima* Willd.) growing on a large estate near Goulds, Florida. A handsome, red, double variety was seriously affected, but the white, pink, and common red, single sorts were free of the trouble. Later, mild cases of the same disease were observed on a strain of red single poinsettia in gardens of Miami, Florida.

Numerous conspicuous, raised lesions or cankers are present on the diseased canes (Fig. 1, A). They are circular to elongate with the longer diameter parallel to the axis of the stem, and range from about 1 mm. to 1 cm. or more in length. Frequently, they become confluent to cover irregular areas, sometimes completely encircling the stem. When this occurs the canes lose their foliage above the girdled area and die back from the tip. Eventually, the centers of individual cankers become somewhat depressed and covered with a grayish to grayish-brown, velvety layer of conidiophores and conidia. The structure of the acervuli and the formation of conidia are typical of fungi belonging to the genus *Sphaceloma*. Microscopic examinations of free-hand and microtome sections of young and old fructifications disclosed no perfect stage of the fungus.

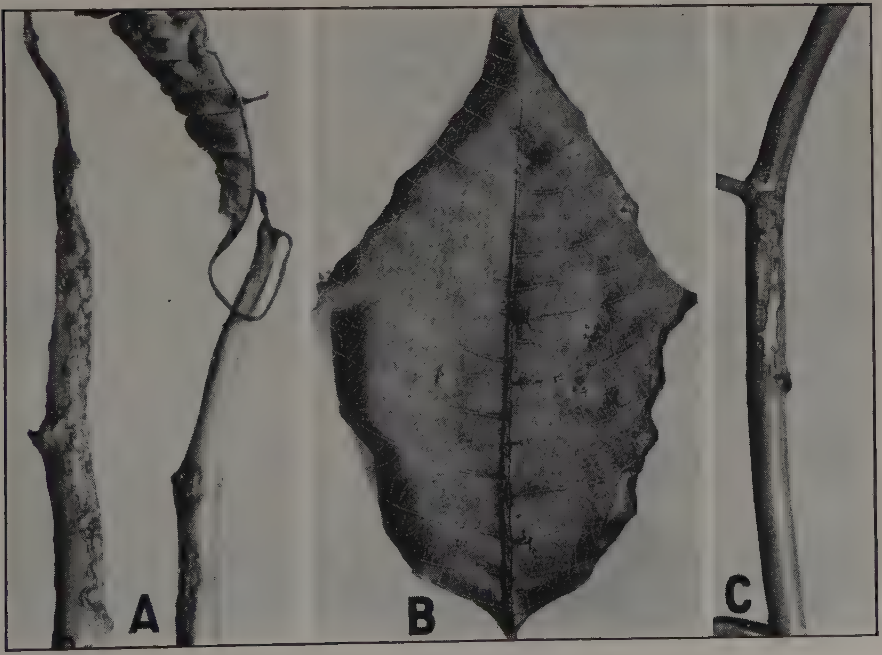

FIG. 1. Poinsettia scab. A. Natural infections on stems and leaf. B. and C. Artificial infections 30 days after inoculation: B. On leaf. C. On stem.

Lesions on leaves are smaller than those on the canes and are confined chiefly to the petioles, midrib, and veins (Fig. 1, B). Except for their usually less pronounced color, they closely resemble scab lesions on citrus leaves caused by *Sphaceloma fawcetti* Jenkins. Affected leaves may be distorted or wrinkled and usually drop prematurely, especially if the petioles become infected.

Tissue plantings on potato-dextrose agar and spore dilutions plated on the same medium gave colonies with a uniform type of growth which resembled in many respects the growth of *Sphaceloma fawcetti* on this medium. Pathogenicity of pure cultures was proved by inoculating young leaves and stems of potted poinsettia plants in September. The method of inoculation was similar to that described by Winston[1] for citrus scab inoculations.

In 7 days infections were readily discernible on both leaves and stems; 30 days after inoculation, typical fructifications were produced on these parts (Fig. 1, B and C). Conidia were formed 14 days after inoculation in some of the acervuli.

Inoculations on young expanding leaves of rough lemon seedlings were unsuccessful with the cultures from poinsettia, but inoculations of rough lemon foliage of like age made on the same day from pure cultures of *Sphaceloma fawcetti* produced typical citrus-scab lesions.

A search through the available literature on poinsettia diseases failed to reveal a description of a similar trouble occurring on this host in the United States. According to a communication from Anna E. Jenkins[2] there does not appear to be any disease described on poinsettia that could be caused by *Sphaceloma*.

Specimens of diseased material have been sent to the herbarium of the University of Florida at Gainesville, Florida. Pure cultures of the causal fungus are being maintained in the laboratory for further study.—GEO. D. RUEHLE, Sub-tropical Experiment Station, Homestead, Florida.

An Apparently Undescribed Disease of the Peanut (*Arachis hypogaea*).[1]—In early September, 1940, the writer chanced upon a disease of the peanut that had come to his attention on but one former occasion. On the basis of a somewhat extensive review of the literature, it is apparent that this disease has not been previously described.

The most striking symptoms of the disease appear on the lower surface of leaves as more or less rectangular, deep brown to almost black splotches (Fig. 1, B, and 2, B). With transmitted light these affected areas show through the upper surface of the foliage as mosaic patterns, with here and there definite pin-point, necrotic areas (Fig. 1, A, and 2, A). Symptoms also appear in varying degree on the petioles and young stems.

A majority of the plants showing the above symptoms were pale green to definitely chlorotic, but in several instances typical symptoms appeared on plants of normal color. Many diseased plants were definitely stunted, poorly nodulated, and produced low yields of pods; often, however, the disease was prevalent on otherwise healthy plants.

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[1] Winston, J. R. Citrus scab and its control. U. S. Dept. Agr. Bull. 1118, 39 p., 1923.

[2] Letter of February 21, 1941, from Anna E. Jenkins, U. S. Dept. Agr., Washington, D. C.

[1] Paper No. 84, Journal Series, Georgia Agricultural Experiment Station.

Histologically, the dorsal epidermis and spongy mesophyll of the leaf appear necrotic, the cell walls being dark-brown (nonstained preparations) and the cell contents being slightly discolored to completely disorganized. Here and there, in limited areas, certain portions of the vascular system were partly discolored. In limited areas in the cells of the lower epidermis and spongy mesophyll there were large bodies, probably ergastic in nature, that stained pale violet to dark brown with Cotton Blue stain. The palisade parenchyma presented a "tight-packed" appearance and the chloroplasts were variably chlorotic. Scattered epidermal necrosis was present, corresponding to the pin-point necrotic areas referred to above.

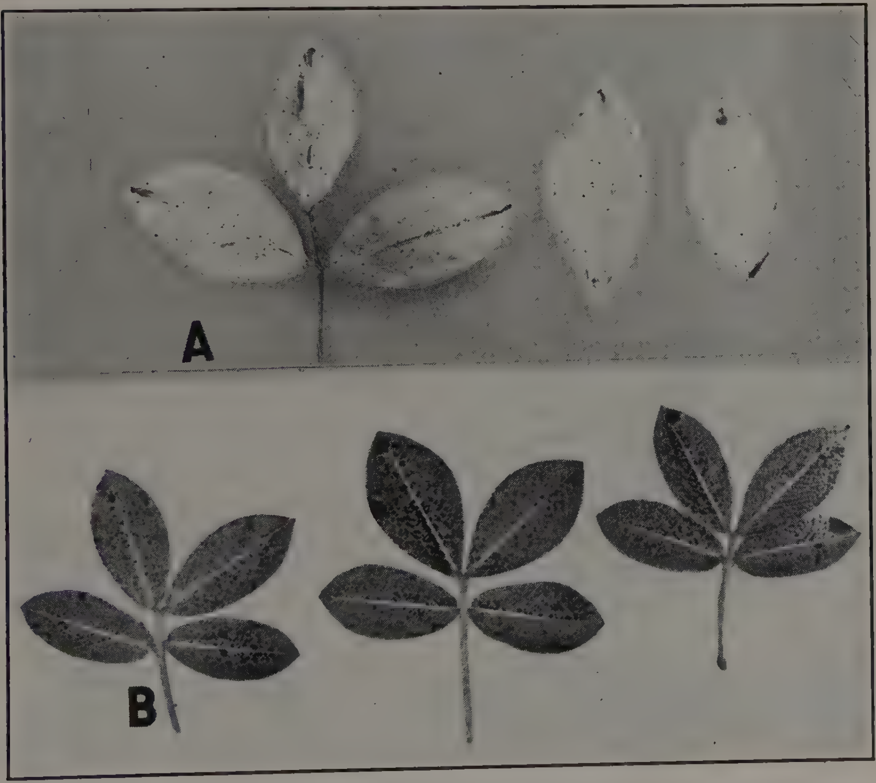

FIG. 1. Symptoms of the peanut disease on the upper (A) and the lower (B) surfaces of peanut leaves collected in the Station plots, Experiment, Ga. (The large spots on the upper left leaflet of the middle leaf and on the upper left and lower right leaflets of the leaf to the right 1, B were produced by *Mycosphaerella arachidicola* Jenks.) × about $\frac{3}{4}$.

All the diseased plants found at Experiment, Georgia, were on heavy clay soil, which had puddled severely following a heavy rain. The writer, however, is reliably informed that the disease appears sporadically in the Coastal Plains areas of Georgia where the soil is sandy. Recently the same disease has been brought to the writer's attention from the vicinity of Yoakum, Texas, and Holland, Virginia,[2] both locations reporting it as rather prevalent in those areas.

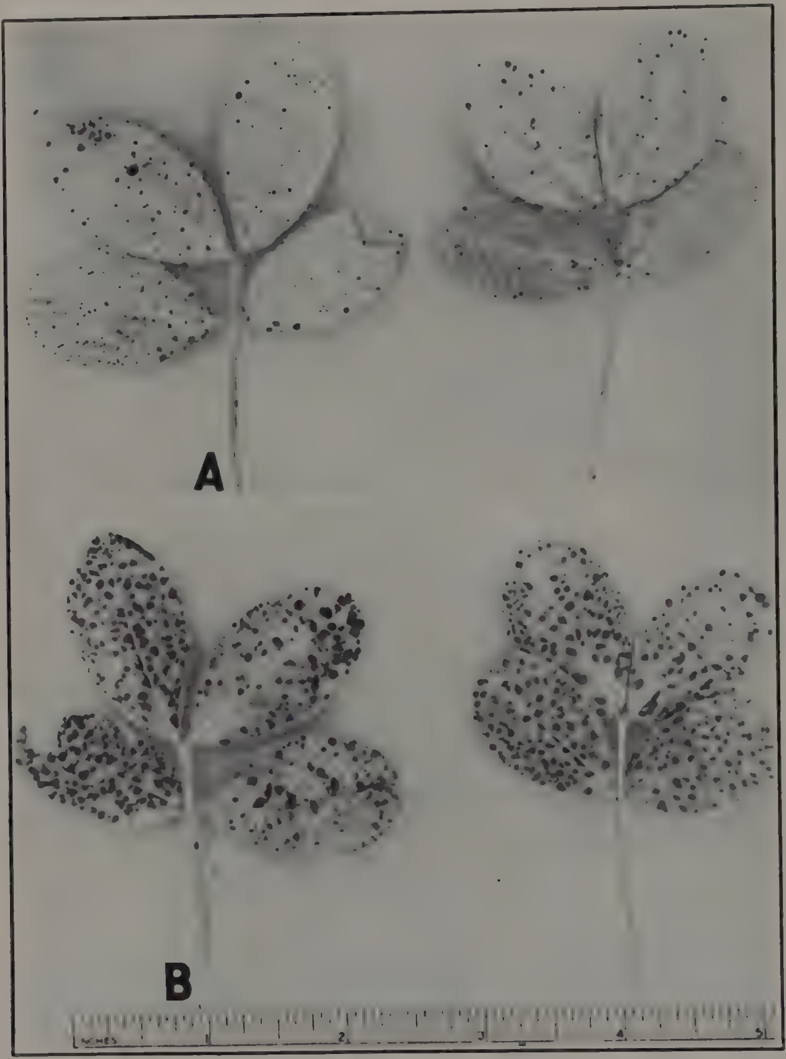

FIG. 2. Symptoms of the peanut disease on a second variety upper (A) and lower (B) leaf surfaces (collected by Dr. A. L. Harrison in the vicinity of Yoakum, Texas). × about ¾.

Results of preliminary work indicate that the disease may be one caused by a mineral deficiency. Repeated attempts to isolate a fungous or bacterial pathogen have failed, as well as several attempts to transmit the disease by the carborundum method. There is no evidence thus far that it is seed-borne. Several types of chlorosis have been found associated with the disease in the fields, but the evidence is strong that some of these may be purely coincidental.

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[2] The writer is indebted to Dr. A. L. Harrison, Plant Pathologist, Tomato Disease Station, Yoakum, Texas, and Mr. L. I. Miller, Assistant Plant Pathologist, Va. Agr. Exp. Stat., Holland, Virginia, for this information.

Several branches from diseased plants, showing severe symptoms, were rooted by the writer and grown by L. C. Olson, of this Station, in complete nutrient solutions in the greenhouse. It was observed that subsequently produced foliage was progressively free from symptoms. The final records on this experiment indicated that the plants had essentially recovered from the disease, as shown by the absence of symptoms on the newer growth. This phase of the work is being continued in the hope that more definite information on the etiology of the disease may be obtained.—WILBERT A. JENKINS, Georgia Experiment Station, Experiment, Georgia.

Fall-Sown Spring Wheat Susceptible to Dwarf Bunt.[1]—Dwarf bunt, caused by a peculiar race of *Tilletia tritici* (Bjerk.) Wint., referred to by Young[2] as a variety, is, in certain years, very destructive of winter wheat in a small area in the northern part of the Gallatin Valley near Bozeman, Montana. In 1940, a few fields were observed in which there was approximately 35 per cent infection, with spots of approximately one-half acre in size in which more than 90 per cent of the heads were infected. The heaviest infection occurred in fields in which the seed had been treated with either copper carbonate or New Improved Ceresan. Since seed treatment with either of these two dusts does not control infection by this race, the use of resistant varieties seems the most satisfactory method of control.

Observations made in farmers' fields show that all the standard winter-wheat varieties grown in the Gallatin Valley are susceptible to dwarf bunt, but no infection in spring-sown spring-wheat varieties has been observed, nor is its occurrence recorded in the literature. Bunt-free seed of 6 varieties of spring wheat and 75 varieties of winter wheat were sown in the fall of 1939 in parts of 2 fields where dwarf bunt had occurred in the previous crop. All 6 spring-wheat varieties survived the winter in 2 replications in 1 nursery and in 1 replication in the other. Based on head counts in 1 replication and estimates in the other 2, the average percentages of infection in the 3 replications were as follows: Pilot, 4; Marquis, 18; Ceres, 18; Reward, 22; Thatcher, 24; and Reliance, 29. The average percentages of infection in 6 winter-wheat varieties were: Turkey, 7; Yogo, 11; Hybrid 128, 17; Montana 36, 23; Karmont, 28; and Kharkof, 41. It is apparent from these experiments that certain spring wheats, as well as winter, are susceptible to dwarf bunt under certain fall planting conditions. Differences in reaction of a spring variety to some of the more common races of the bunt fungi under spring and fall sown conditions have previously been reported.[3, 4]—R. H. BAMBERG, Assoc. Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, Agricultural Experiment Station, Bozeman, Montana.

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[1] Journal Paper No. 145 of the Montana Agricultural Experiment Station, Bozeman, Mont. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Montana Agricultural Experiment Station.

[2] Young, P. A. A new variety of *Tilletia tritici* in Montana. Phytopath. 25: 40. 1935.

A Satisfactory Medium for Germination of Urediospores of Puccinia graminis tritici.[1]—In the course of routine work at the Federal rust laboratory at University Farm, St. Paul, Minnesota, a method that gives quick and consistent results has been developed for the germination of urediospores of *Puccinia graminis tritici.* Older methods did not seem satisfactory because of the variability often noted between replicates within a test and between tests. The following are some of the factors that might account for this variability: Unclean glassware, the difficulty of obtaining a uniform distribution of spores when these are dusted on the surface of water or agar, uneven wetting of the spores, the effect of surface tension in small drops of water, inability to observe doubtful cases of germination under the high-power objective of the microscope, and the difficulty of observing spores floating about on an unstable water surface.

Not all these causes or accompaniments of variability in germination are encountered in any one method, but most of them would seem to result from the use of water as a germinating medium. Consequently, solid media, including glycerin jelly, three concentrations of gelatine, and 7 concentrations of water agar were tested in preliminary experiments. It was found that 1-per cent distilled-water agar served the purpose best when used in the following manner: Ten cc. of melted 1-per cent water agar is poured into a Syracuse dish and allowed to harden. The spores under test are then mixed in a drop or two of distilled water on a clean, hollow-ground glass slide, and one or two loopfuls of this suspension are transferred to the surface of the agar. The drops are thoroughly spread about with the flat side of a small bent spatula. In order that the spores may be kept in a limited area where they may be easily found and observed, only the center of the agar surface is utilized. The dishes should not be stacked or covered with airtight covers, as variable results are sometimes obtained with dishes so treated. In the noncovered dishes adequate moisture is available for spore germination, which can be easily noted at the end of 2 or 3 hours at room temperature.

This method has proved advantageous for the germination of urediospores of *Puccinia graminis tritici* for several reasons, as explained below.

(1) The results were consistent to a high degree. In germination tests of 26 collections of fresh urediospores, each replicated 5 times, the greatest difference in germination between the 5 replicates of any one test was 12 per

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[3] Heald, F. D. The control of bunt or stinking smut of wheat. Wash. Agr. Exp. Stat. Bull. 241, 30 pp. 1930.

[4] Smith, W. K. The effect of different temperatures on the reaction of Hope wheat to bunt. Phytopath. 22: 615–627. 1932.

[1] Cooperative investigations between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station. Published with the approval of the Director as Paper No. 1878 of the Minnesota Agricultural Experiment Station.

cent, the lowest was 1 per cent, and the average was 5.4 per cent. The average germination of these 26 lots of urediospores was 95 per cent. In a second experiment 1 lot of urediospores was kept in the laboratory, and germination tests were made of these spores at various intervals. The germination percentage gradually decreased over a period of 1½ months, but in the 15 germination tests the greatest difference between the 4 replicates of any one test was 17 per cent, the lowest was 1 per cent, and the average was 6.7 per cent. As a comparison, tests were made with water as the germinating medium in a Syracuse dish. Not only were the germination percentages lower, but the differences between the 4 replicates of 1 test rose as high as 39 per cent and the average difference was 19.2 per cent.

(2) Viability of old urediospores can be determined more accurately than by other methods. Of urediospores kept in the laboratory for 1½ months, 5 per cent germinated on water agar, whereas no germination could be detected in a water medium.

(3) The trouble caused by chemically unclean glassware was eliminated. Syracuse dishes were dipped in bichloride of mercury and then washed in tap water. One-per cent water agar was poured into half of these dishes and distilled water into the others. Spores germinated on the agar normally, whereas, on the water, germination was very erratic.

(4) Germination is easily noted because spores are evenly distributed and so separated that they are readily observed individually with the low-power objective of the microscope. If it is doubtful whether certain spores have germinated they can be observed with the high-power objective without difficulty.

It is possible that this method may be useful also in similar studies on other fungi.—WILLIAM Q. LOEGERING, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, University Farm, St. Paul, Minnesota.

Spore Germination of Selenophoma bromigena.—While studying the cultural characters of *Selenophoma bromigena* (Sacc.) Sprague and Johnson,[1] which causes a leaf spot of *Bromus inermis,* it was observed that the type of spore germination differed from that previously reported.[2] The writer, therefore, has studied germination in different collections of the fungus.

As previously reported, when spores germinate in sterile distilled water, a germ tube develops from one end of the spore (Fig. 1, A), or, occasionally, from both ends, and the original spore remains visible for some time. On potato-dextrose agar, however, the process is different. Eleven to 15 hours after being placed on agar the spore swells slightly and 1 or 2 septa are formed. Swelling occurs mostly at the ends, but is not restricted to them. Within the next few hours germ tubes develop from both ends of the spore and more septa appear within the original limits of the spore and also in the terminal germ tubes. Lateral germ tubes may arise from newly formed cells of the original spore as soon as the septa are formed. As many as 3 hyphae have been observed to arise from one cell, while some cells never produce any. After germination, the spore is no longer recognizable as it is when germinating in sterile water (Fig. 1, B, C, and D).

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[1] Sprague, R., and A. G. Johnson. Selenophoma on grasses. Mycologia 32: 415. 1940.

[2] Unpublished data in a Ph.D. thesis presented to the University of Minnesota by J. Lewis Allison.

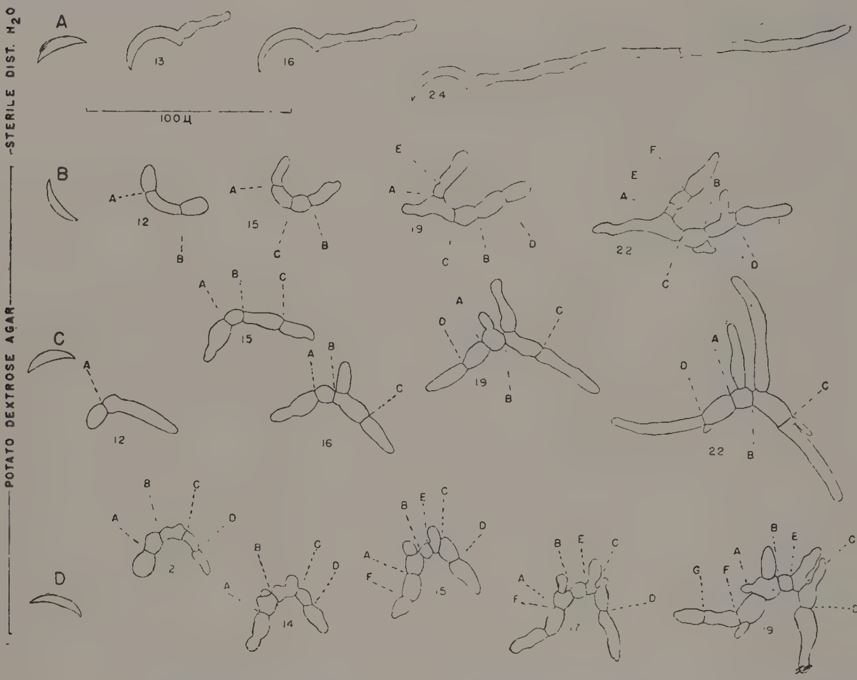

FIG. 1. Camera-lucida drawings of 4 germinating spores of *Selenophoma bromigena*. The number under each spore indicates the time in hours that it has been germinating. The letters serve to identify the septa during the process of germination.

This formation of septa during germination has been observed in more than 300 single-spore isolates and many other mass-spore isolates in collections of the fungus from Canada, North Dakota, South Dakota, and Minnesota.

The effect of various media and temperatures on the type of germination was studied. Spores were germinated in sterile distilled water, Richards solution, potato decoction, and on water agar, malt agar, and on potato-dextrose agars containing different concentrations of dextrose and agar. The "septation type" of germination occurred on all media except sterile distilled water. Spores were germinated on standard potato-dextrose agar at 10, 15, 20, 25, and 30 degrees C., and germination was of the "septation type" at all temperatures.—ELLIS F. DARLEY, University Farm, St. Paul, Minnesota.

The Use of Iodine in the Control of Potato Ring Rot and Scab.—Iodine, one of the strongest disinfectants, has been used experimentally for controlling certain plant diseases. Sayre and Thomas,[1] reported excellent results on the control of oat smuts with iodine-vapor dust. Young and McClelland,[2] working with a 10 per cent solution of iodine in carbon bisulphide, obtained variable results in attempting to control oat smuts. An alcoholic-iodine solution was successfully applied for the control of *Diplodia natalensis* on orange by Reichert and Littaver.[3] Raleigh[4] found that 0.25 per cent potassium iodide added to a 1:1200 mercury bichloride solution increased the efficiency of the latter as a dip for seed potatoes in the control of *Rhizoctonia solani* and that a 2 per cent solution of potassium iodide used alone did not control this fungus. No injury was noted.

In the course of studies in California on the bacterial ring rot of potatoes, caused by *Phytomonas sepedonica,* iodine was tried as a dip for cut seed pieces and as a cutting-knife disinfectant to prevent the spread of bacterial ring rot and as a dip for uncut seed potatoes to control scab and *Rhizoctonia solani.*

Considerable seed-piece injury and reduction in germination resulted when cut seed pieces were dipped for 3 minutes in a solution of 0.5 per cent iodine in 1 per cent potassium iodide, even when the seed pieces were rinsed in water after treatment. This treatment is injurious to seed-pieces and is not effective against ring rot.[5,6] However, the disinfection of the cutting knife with a 1 per cent solution of iodine considerably reduced the amount of ring rot in the new crop. In the experimental tests, a ring-rot-infected potato tuber was cut, the slime adhering to the knife was wiped off with a cloth, and the knife was then dipped momentarily in the iodine solution before cutting each healthy tuber.

In a field test at Shafter, using the White Rose variety, 5.3 per cent of the 225 hills were infected where the disinfected knife had been used, and the stand was 92 per cent. Where the knife was not disinfected, 78.7 per cent of the 169 hills were infected and the stand was only 71 per cent.

For knife disinfection, an effective formula is 38 g. iodine, 76 g. potassium iodide, 1 pt. of glycerine and 2 gal. of water. The knife should be wiped on a cloth prior to dipping in order to insure the removal of masses of bacteria that might otherwise be protected by slime and tissue débris from actual contact with the disinfectant.

Laboratory tests showed that the scab organisms within the scab lesions were killed by a 5-min. immersion of infected tubers in a 1 per cent iodine solution. Greenhouse tests showed that no injury was caused by this treatment when applied to whole tubers.

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[1] Sayre, J. D., and R. C. Thomas. New dust treatments for oat smuts. Science (n.s.) 28: 858–859. 1938.

[2] Young, V. H., and C. K. McClelland. Control of oat smuts. Phytopath. 23: 825–830. 1933.

[3] Reichert, I., and F. Littaver. A new method of control of wastage in oranges. Hadar 10: 1–13. 1937.

[4] Raleigh, W. P. Fungicidal efficiency of a solution of mercuric chloride and potassium iodide. (Abstract) Phytopath. 23: 28–29. 1933.

[5] Kendrick, J. B., and C. Emlen Scott. Summary of potato ring rot experimental plots in California. 17 p. California Agr. Exp. Stat. (Mimeograph). 1940.

[6] Kendrick, James B., P. A. Ark, and C. E. Scott. Potato ring-rot studies in California. (Abstract) Phytopath. 31: 16. 1941.

In a field test at Shafter on the effectiveness of this treatment on whole Russet Rural seed potatoes heavily infected with scab, 2.5 per cent of the tubers grown from the treated seed showed scab, as compared with 47 per cent tuber infection in the control rows. In a similar field test at Davis, in which whole tubers were treated for 1 minute, 8 per cent of them showed scab infection as compared with 69 per cent in the control rows.

The 5-min. treatment, as applied at Shafter to Russet Rural seed tubers, bearing sclerotia of *Rhizoctonia solani,* failed to control this disease.

Further tests with the 5-min. treatment of whole tubers showed that no reduction of germination was caused, even after the treated tubers were stored 5 months.

Thus it appears that iodine may be useful as a disinfectant for the cutting knife to reduce the spread of ring rot and for whole seed tubers to reduce the incidence of scab.—P. A. ARK, Division of Plant Pathology, University of California, Berkeley, California.

Chemical Eradication of Crown Gall on Almond Trees.—Crown gall (*Phytomonas tumefaciens* (Sm. and Towns.) Bergey *et al.*) kills annually many productive orchard trees because of lack of reliable methods of controlling the disease in its early stages. In certain sections of California it is so serious that it has become a limiting factor in growing almonds and peaches.

Attempts by both growers and investigators to eradicate the galls with Bordeaux-mixture paste or creosote compounds has resulted either in complete failure to kill the galls or in the death of treated plants. Although accumulated evidence shows that crown gall may be avoided by growing plants in acid soils,[1, 2] this is of more importance to nurserymen than to orchardists, because nearly all infections of orchard trees are attributable to mechanical injury at the crown followed by contamination of the wounds with soil infested with *Phytomonas tumefaciens.* Thus the need for a good disinfectant for the eradication of the galls is obvious.

Of the many chemicals tested by the writer during the past 3 years in seeking a method of eradicating the galls, sodium dinitro-cresol (sold as Elgetol), iodine, and clove oil gave the most satisfactory results.

Sodium dinitro-cresol mixture was prepared by taking 20 volumes of Elgetol and 80 volumes of methanol (methyl alcohol). The mixture is shaken well before using, and can be painted on a clean gall, covering thoroughly the surface of the gall with a suitable painter's brush and extending the application about ½–1 inch beyond the outlines of the gall of healthy bark.

Of the iodine solutions, the following formulae gave very satisfactory results: Solution A.—Methanol, 50 parts; acetic acid (glacial), 25 parts;

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[1] Siegler, E. A. Relation between crown gall and pH of the soil. Phytopath. 28: 858–859. 1938.

[2] Stapp, C., and H. Müller. Der Pflanzenkrebs und sein Erreger *Pseudomonas tumefaciens.* VII. Mitteilung Untersuchungen über die Möglichkeit einer wirksamen Bekämpfung an Kernobstgehölzen. Zentralbl. f. Bakter., etc. Abt. 2. **99**: 210–276. 1938.

glycerine, 25 parts; and iodine (metallic), 10 parts. Solution B.—Methanol, 100 parts; acetic acid, 15 parts; and iodine (metallic), 12 parts. Solutions A and B are designed for use on whole galls. For applying these materials on a scarified surface, solutions A and B must be diluted with methanol 5 and 6 times, respectively.

The clove oil appears very effective in combination with methanol and glacial acetic acid. A formula, used experimentally by the writer, was as follows: Clove oil, 1 part; glacial acetic acid, 1 part; and methanol, 2 parts. In the event that a grower prefers to cut off the gall and treat the wound, the clove oil mixture should be diluted five times with methanol and metallic iodine added to make a 2 per cent strength. Clove oil is costly, and it is doubtful if it will ever prove of practical value in treating crown gall. Of the chemicals mentioned, sodium di-nitro-cresol (Elgetol) mixture is the cheapest and can be applied to the galls at any time of the year under California conditions.

Tests showed that sodium di-nitro-cresol (Elgetol)-methanol mixture gave 100 per cent control of the galls of almonds, ranging in size from 3 to 10 inches in diameter. On almond trees, 55 galls treated from July to December, 1939, were found dead when checked in May, 1940, and no new tumor tissue was observed around the remnants of the treated galls in September, 1940.

Iodine and clove-oil solutions are most effective when applied to galls in summer. Galls treated in hot weather were dead within 2 to 3 months. Table 1 shows the behavior of the galls treated with Solution A and the season of the year when treated.

TABLE 1.—*Effect of Solution A on crown galls of almonds treated in San Joaquin Valley, California*

Month	No. galls treated	No. galls killed after 2 months	No. galls killed after 6 months	No. galls killed after 12 months
June–July	20	14	18	20
September	18	15	16	18
December	15	0	7	12

In some cases it was possible to eradicate the gall when it had almost completely girdled the tree. In such cases, the treatment must be performed only on part of a gall at one time. It is dangerous to paint such large galls with the chemicals mentioned above, since their accumulation in the plants may become sufficiently great to cause death.—P. A. ARK, Division of Plant Pathology, University of California, Berkeley, California.

Observations on the Epidemiology of Tobacco Wildfire and Blackfire.—The relation of water-soaking of leaves to the infection and development of tobacco wildfire and blackfire (*Bacterium tabacum* Wolf and Foster, and *B. angulatum* Fromme and Murray) has been recognized and described by

Clayton[1] and Johnson.[2, 3] Since the "internal" or physiological type of water-soaking has been observed on many species of plants under natural conditions, it is of general interest to know more about the factors contributing to its occurrence. Repeated efforts in this laboratory over a period of several years to reproduce the physiological type of water-soaking at will by artificial variations of the environment have not been entirely satisfactory.[2] Recently, however, an important determining factor, namely, light intensity, has been demonstrated. When tobacco (*Nicotiana tabacum* L.) plants grown in a sandy soil in the greenhouse were placed in a moisture-saturated atmosphere, typical and abundant water-soaking of the leaves was obtained in darkness or in low light intensity (Fig. 1). Higher intensities of light, solar or artificial, prevented water-soaking under identical conditions of temperature and humidity. The reason for this response is not entirely clear, although it is assumed that it is related to transpiration. Since the water-soaked condition must be retained for several hours to permit infection,[3] the time of day when it is initiated and the amount of sunlight may often determine the occurrence and severity of the disease.

Some epidemics of the bacterial diseases in question apparently are not associated with any definitely known source of infection. This has led some investigators to believe that the parasites regularly evolve from certain common soil saprophytes,[4] or that the parasites overwinter in the soil.[5] As possibly bearing on these theories, it has been found in this laboratory that a great variety of plants, including some common crops, may be infected by wildfire and blackfire organisms when in the water-soaked condition brought about by either internal or external forces.[3] It has now been found that the bacterial parasites in question may survive as long as 6 months in dead leaves of such atypical hosts as alfalfa (*Medicago sativa* L.) hay, refuse from beans (*Phaseolus vulgaris* L.), ragweed (*Ambrosia bidentata* L.), lambsquarter (*Chenopodium album* L.), and many other plants, as well as tobacco, when stored in the dried condition. The opportunity for the organisms to overwinter and be disseminated in such material is, therefore, very good. This is not contradictory to Clayton's[6] earliest results, since, at that time, the relation of water-soaking to infection was unknown and the multiplication of the bacteria in the tissues of many hosts other than tobacco was not readily obtained by artificial means. It is planned to publish the detailed results of these investigations at a later date.—WILLIAM B. ALLINGTON,

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[1] Clayton, E. E. Watersoaking of leaves in relation to development of the wildfire disease of tobacco. Jour. Agr. Res. [U.S.] 52: 239–269. 1936.

[2] Johnson, J. Relation of root pressure to plant disease. Science 84: 135–136. 1936.

[3] Johnson, J. Relation of water-soaked tissues to infection by *Bacterium angulatum* and *Bact. tabacum* and other organisms. Jour. Agr. Res. [U.S.] 55: 599–618. 1937.

[4] Reid, J. J., J. Naghski, R. G. Harris and H. D. McAuliffe. A cultural and serological study of *Phytomonas tabaca* and certain related forms. Jour. Bact. 37: 234–235. 1939.

[5] Valleau, W. D., and S. Diachun. Burley tobacco diseases in Central Kentucky in 1940. Pl. Dis. Rptr. 25: (1, p. 18). 1941.

[6] Clayton, E. E. Toxin produced by *Bacterium tabacum* and its relation to host range. Jour. Agr. Res. [U.S.] 48: 411–426. 1934.

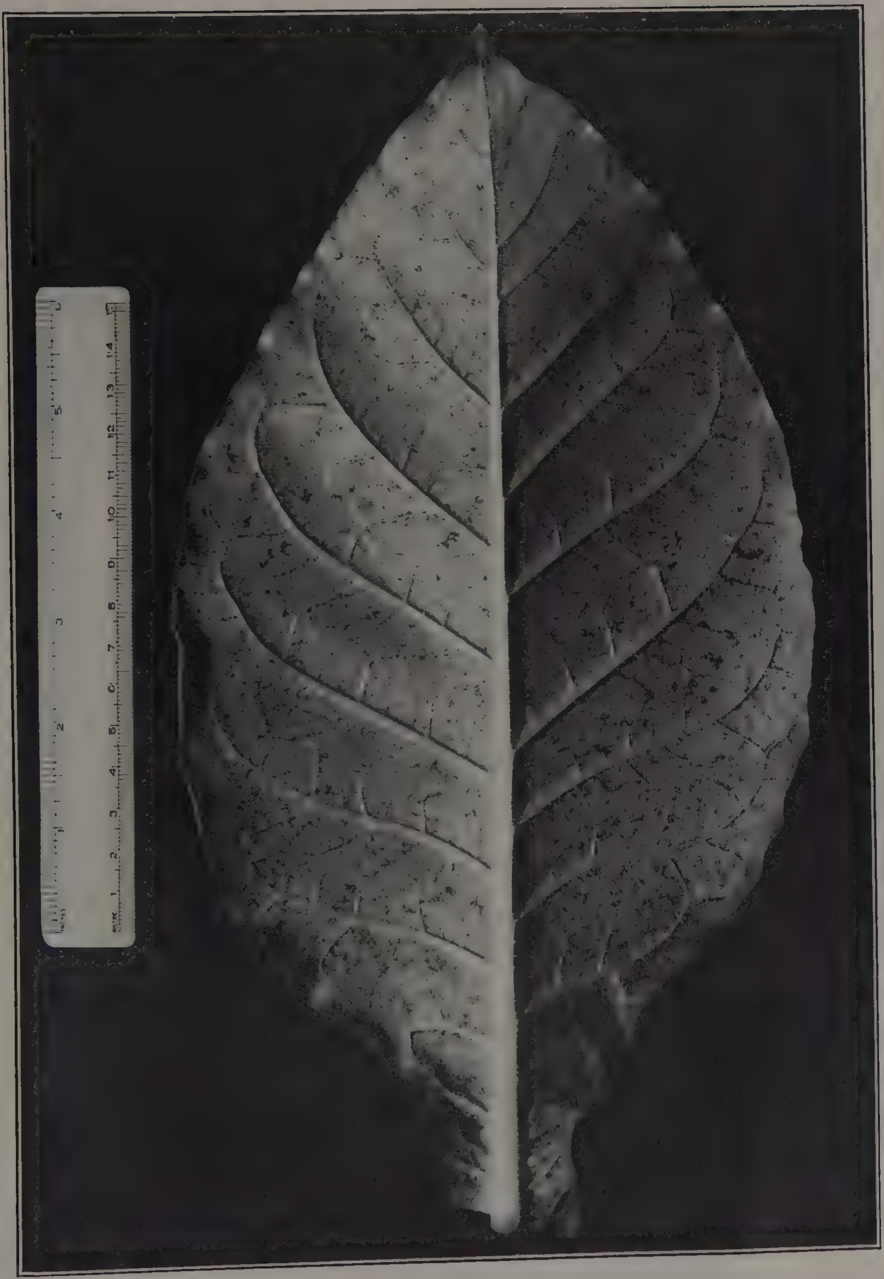


FIG. 1. Water-soaked areas on a large leaf secured under reduced light intensity. Other conditions being the same, light appears to be a controlling factor.

Division of Tobacco Investigations, U. S. Department of Agriculture, Washington, D. C., in cooperation with Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

BOOK REVIEW

Ciferri, Raffaele. *Manuale di Patologia Vegetale.* (Manual of Plant Pathology.) S. Lapi Publishing House. (Cittá di Castella) Italy, 1941.

This is probably the most concise and comprehensive book on plant pathology yet published by an Italian. It differs greatly from most of the previously published works in that the author does not fill it with life histories of pathogens. He does, of necessity, include some of this material but stresses (what is more valuable) the control of diseases by adjusting environmental and cultural conditions. Ciferri feels that more is accomplished by a detailed treatment of relatively few diseases involving fundamental problems, than by a superficial treatment of a large number of diseases.

Although the book is written primarily for Italian students in plant pathology, a careful perusal reveals that it presents a more comprehensive view on all the problems facing plant pathologists than many similar books in other languages. Italian investigators are cited almost exclusively, as the author says, "to acquaint Italian students with the large number of Italian investigators in the field." Consequently, the book gives what is probably the best coverage of Italian publications ever attempted. Other workers are cited only where some fundamental work by non-Italians is essential to emphasize or clarify the point under discussion.

Manuale di Patologia Vegetale is divided into 7 parts; the length of each part being a fair indication of its relative importance to the field as a whole.

Part one (122 pages) deals with generalities. In it are included excellent discussions on disease concepts, ecology as it affects parasitic diseases, classification of diseases, predisposition, susceptibility, resistance and immunity, methods of dissemination of parasitic agents, natural enemies of plant pathogens, and control measures. The use of resistant varieties, disease prevention by cultural practices, and fungicides are treated in considerable detail under control measures. The discussion on fungicides, for example, is treated more completely and is more timely than any Italian book on plant pathology I have ever seen. Seed disinfection, soil disinfestation, and disease symptoms complete the first section.

The information in part two on nonparasitic diseases is presented in sufficient detail to make this section one of the most valuable in the book. The treatment of diseases resulting from inadequate or excessive light, unfavorable temperatures, humidities, and soils, and from inert dusts, toxic gases, and spray materials, are helpful not only to students but to advanced workers, as well. Approximately 100 pages of valuable information are found in this section.

Part three (80 pages) covers virus diseases rather well. The diseases are sufficiently well illustrated to give the student a good idea of the varied symptoms produced by viruses. The more important researches of non-Italian investigators are covered in greater detail here than in any other part of the book.

Part four (42 pages) discusses diseases caused by cryptogamic parasites. Here, the pattern follows closely that used by other Italian authors, although Ciferri is more explicit than most, and appears to be a good evaluator of the more important contributions in the field. His selection of typical diseases exemplifying rather large groups is good.

The club-root organism on Cruciferae and its control, as well as related parasites, are discussed in the 5 pages of part five.

Part six (287 pages) includes diseases caused by Eumycetes. The treatment here follows previously published Italian books more closely than any other section in the book.

Phanerogamic parasites are discussed in considerable detail in the 40 pages of part seven. A number of appendices are included, all of which help to enhance the value of the book. Among the subjects covered in these sections are: The morphological and biological characteristics of fungi and their significance, sexuality in fungi, variability in fungi, harmful algae, and weed control.

The 256 illustrations were taken largely from previously published Italian literature. These were apparently chosen with care, inasmuch as they illustrate the diseases under discussion rather well. The book is inexpensive mainly because of the type of paper and binding used and not because of the contents.—P. P. Pirone, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

WILLIAM WILLIAMS GILBERT
1880–1940

L. L. HARTER

William Williams Gilbert, senior pathologist, Bureau Plant Industry, United States Department of Agriculture, died in Washington, D. C., December 22, 1940.

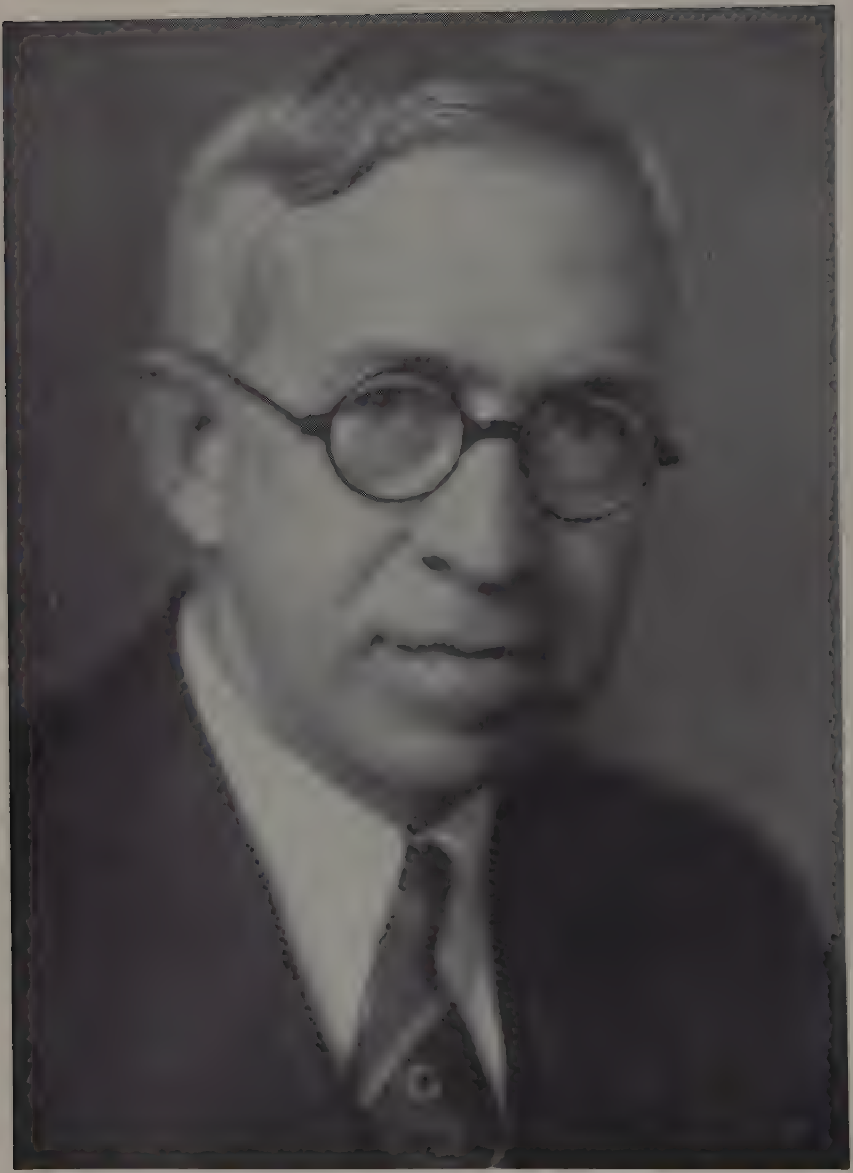
He was born at Dorset, Vermont, and spent his childhood days on a farm. His elementary education was obtained in the public school of Bennington County, and his college preparatory work at Troy Conference Academy, Poultney, Vermont, which he attended from 1896 to 1900. In the fall of 1900 he entered the University of Vermont from which he graduated with the B.S.A. degree in 1904, with Phi Beta Kappa honors. During a portion of his undergraduate study, he was an assistant in the department of botany. In 1909 he was awarded the M.S. degree from the University of Vermont in plant pathology. Postgraduate work also was done at the University of Wisconsin, George Washington University, Columbia University, and in the Graduate School of the United States Department of Agriculture.

Mr. Gilbert was a member of the following scientific societies: Botanical Society of Washington, Washington Academy of Science, Fellow A.A.A.S., American Phytopathological Society (charter member), Botanical Society of America, and Vermont Botanical Society.

In 1904, Mr. Gilbert was appointed a scientific assistant in the office of Cotton and Truck Crop Diseases in the Bureau of Plant Industry, to assist the late Dr. W. A. Orton in breeding cotton, watermelons, and cowpeas resistant to disease. During the period of this work he spent much of his time in some of the Southern States, and assumed much of the responsibility for the breeding trials and in the general carrying out of the program. Later he conducted investigations on certain of the diseases of tobacco and cucumbers.

His entire scientific career was spent in the U. S. Department of Agriculture. He was advanced from scientific assistant to assistant pathologist in 1909, to pathologist in 1913 and to senior pathologist in 1928.

His scientific investigations were interrupted at the height of their productiveness in 1930 to fill a call in the Division of Horticultural Crops and Diseases for a man with a thorough scientific background to assume certain editorial responsibilities with respect to manuscripts originating in the Division. His duty was to see that the manuscripts were in the proper form for publication in the various journals and that the articles did not contain subject matter for which the Department should not assume responsibility. Mr. Gilbert proved to be the right man for the job. Being gifted with a flair for detail and thoroughness, the possessor of a spirit of helpfulness accompanied by an infinite supply of tact, he was able to deal successfully with the most sensitive authors. Mr. Gilbert served in this capacity until



WILLIAM WILLIAMS GILBERT

his death. Although in poor health for a number of years, he faced his problems and the future with courage and discharged his obligations without complaint.

He was a noble Christian gentleman in every sense of the word—a man who seemed to see good in everyone and everything. Those who knew him were the better for it and those who knew him the longest and best, appreciated him most. The possessor of a kindly, tolerant, sympathetic, and even disposition, he greeted everyone cordially, despite the physical handicaps resulting from many years of illness.

Publications that bear Mr. Gilbert's name as author or co-author, are:

PUBLICATIONS OF WILLIAM W. GILBERT

The root-rot of tobacco caused by *Thielavia basicola*. U. S. Dept. Agr., B. P. I. Bull. 158. 1909. (At request of Italian government, this bulletin was translated and published in Italian and distributed by the Italian government to Italian tobacco growers.)

A method of inbreeding cotton. Proc. American Breeders Assn. 8: 405–409. 1912.

The control of cotton wilt and rootknot. U. S. Dept. Agr., B. P. I. Circ. 92. 1912.

Cotton anthracnose and how to control it. U. S. Dept. Agr. Farmers' Bull. 555. 1913.

Cotton wilt and rootknot. U. S. Dept. Agr. Farmers' Bull. No. 625. 1914.

Lightning injury to potato and cotton plants. Phytopath. 5: 94–102. 1915.

Cucumber mosaic disease. Phytopath. 6: 143–144. 1916.

Seed treatment control and overwintering of cucumber angular leaf spot. Phytopath. 8: 229–233. 1918.

Lightning injury to herbaceous plants. Phytopath. 8: 270–282. 1918.

Boys and girls club work: diseases and insects of the home garden. U. S. Dept. Agr. Circ. 35. 1919.

Seed transmission of cucurbit mosaic by the wild cucumber. Phytopath. 9: 326–327. 1919.

Soil disinfection by hot water to control the rootknot nematode and parasitic soil fungi. U. S. Dept. Agr., B. P. I. Bull. 818. 1920.

Cotton diseases and their control. U. S. Dept. Agr. Farmers' Bull. 1187 (superseded in 1935 by F. B. 1745). 1921.

Field tests with cucumber angular leaf spot and anthracnose. Phytopath. 11: 298–299. 1921.

Diseases and insects of garden vegetables. U. S. Dept. Agr. Farmers' Bull. 1371. 1924.

Diseases and pests of fruits and vegetables. U. S. Dept. Agr. Yearbook (1925): 453–599. 1925.

An improved method for isolation of *Thielavia basicola*. Phytopath. 16: 579. 1926.

Control of mosaic and spot diseases affecting cucumbers. Canner 64: (10). 189–190. 1927.

Control of cucumber and cantaloup diseases in Maryland. Report of Maryland Vegetable Growers' Assn. (1928): 400–413. 1928.

Chemical injury to watermelon in transit. U. S. Dept. Agr. Circ. 74. 1929.

William A. Orton. (A biographical note.) Science 71: 89–91. 1930.

Jesse Baker Norton. (A biographical note.) Jour. Hered. 31 (6): 273–276. 1940.

COMPARATIVE PHYSIOLOGY OF CROWN GALL, ATTENUATED CROWN GALL, RADIOBACTER, AND HAIRY ROOT BACTERIA[1]

A. J. RIKER, MARY M. LYNEIS, AND S. B. LOCKE[2]

(Accepted for publication March 15, 1941)

INTRODUCTION

Some basic questions concerning atypical and pathological growths as approached through studies on plants, and particularly on the crown-gall disease, have been reviewed by Riker and Berge (9). As they have explained, one approach to these problems is through a comparative study of pathogenic and attenuated crown-gall bacteria. Another correlated approach is through a study both of the pathogenic culture and of the tomato host in relation to the critical temperature range (28° to 30° C.) below which crown gall is found on inoculated tomato plants and above which no crown gall develops. These two lines of inquiry have been followed through certain investigations on nitrogen and carbon metabolism, physical chemical changes induced, serological reactions, and host-parasite relations.

METABOLISM STUDIES

The cultures used were all progenies of mechanically picked single cells. Two cultures of *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.*, the cause of crown gall, have been employed, one pathogenic (A6, Wright *et al.*, 14) and one attenuated (A6–6, Hendrickson *et al.*, 3), the attenuated culture being a single-cell isolation from the mother culture. The culture (C 10) of *Phytomonas rhizogenes* R.B.W.K.S., the cause of hairy root, and the never pathogenic culture of *Bacillus radiobacter* Beij. and Van Deld. (R–2), used occasionally for comparison, were also derived from single-cell isolations. From time to time during these studies the cultures were inoculated into plants to check their pathogenicity.

Unless otherwise noted all the determinations reported were made in duplicate or triplicate, and were carried out 3 separate times.

Utilization of Nitrogen

Both organic and inorganic sources of nitrogen have been studied in regard to their utilization by pathogenic and attenuated crown-gall cultures. Since the hairy-root culture does not utilize nitrate as a sole source of nitrogen, it is not included in the ensuing data. The Radiobacter culture was employed as a further check on the crown-gall cultures because of the occasional physiological differences reported by Sagen *et al.* (10) between it and *Phytomonas tumefaciens.* Except for pathogenicity, they seem very closely related.

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[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by the International Cancer Research Foundation.

[2] The writers are indebted to Eugene Herrling for aid in preparing the illustrations.

The medium used contained: magnesium sulphate ($MgSO_4 \cdot 7H_2O$), 0.2 g.; sodium chloride (NaCl), 0.2 g.; calcium chloride ($CaCl_2$), 0.1 g.; dibasic potassium phosphate (K_2HPO_4), 0.2 g.; potassium nitrate (KNO_3), 5.0 g.; glucose, 5.0 g.; and distilled water to make 1000 cc.

The method of seeding culture tubes was standardized as follows: The bacteria were grown on the very favorable yeast-infusion-potato-dextrose agar. The potatoes were sliced into water and autoclaved for 20 minutes at 15 pounds' pressure, and the unoxidized filtrate was used in the medium. After 48 hours' incubation at room temperature, the bacteria were mixed with sterile distilled water to form a suspension corresponding in density to McFarland's (4) 0.5 per cent barium sulphate nephelometer. It was added at the rate of 0.2 cc. into each tube containing 10 cc. of medium. The cultures were incubated, respectively, at 28° and 32° C.

Cultural characteristics were observed over a period of 3 weeks. All the pathogenic and attenuated crown-gall bacteria incubated showed abundant growth and formed pellicles. Radiobacter, however, failed to form a pellicle.

The utilization of the nitrate included in the medium was studied by tests for nitrates, nitrites, and ammonia, by tests with diphenylamine, Trommsdorf's reagent, and Nessler's reagent, respectively. The hydrogen-ion concentrations induced by these organisms were measured with a quinhydrone electrode, so the results are only approximate in the strongly alkaline ranges. Representative results appear in table 1. Radiobacter used up all the nitrate with a strongly alkaline reaction. Doubtless some nitrate was employed by the other bacteria in building protoplasm, but tests for nitrite and ammonia were negative.

TABLE 1.—*Changes after 3 weeks in reaction and in nitrate utilization by specified bacteria grown at given temperatures*[a]

Culture	Incubated at 28° C.				Incubated at 32° C.			
	pH	NO_3	NO_2	NH_3	pH	NO_3	NO_2	NH_3
Pathogenic crown gall	7.3	++	–	–	7.8	++	–	–
Attenuated crown gall	6.9	++	–	–	6.9	++	–	–
Radiobacter	8.7	–	–	±	8.9	–	–	±
None	7.4	+++	–	–	7.4	+++	–	–

[a] Reactions are indicated as follows: +++ strong, ++ moderate, + slight, ± trace, and – negative.

Aspartic acid, glutamic acid, and tyrosine were used, both alone and in combination, as sources of nitrogen. The basic salt medium described earlier was used. The amino acids alone or in a mixture were substituted for the 5 g. of potassium nitrate. After seeding, the cultural characteristics were studied at both 28° and 32° C. for a 2 week's incubation period. Hydrogen-ion determinations were run on the cultures, and tests were made for the presence of nitrates, nitrites, and ammonia.

A mixture of the 3 acids was a good source of nitrogen for pathogenic and attenuated crown gall and for Radiobacter organisms. Both strains of crown gall produced pellicles and a yellow color in the liquid media. Radiobacter produced a pellicle, but the media showed no color. Hairy-root cultures showed only slight turbidity and no pellicle formation or color. Readings for hydrogen-ion concentration and for the presence of nitrate, nitrite, and ammonia showed no distinctive difference in cultures incubated above and below the critical temperature range. All cultures, except hairy root, induced an alkaline reaction and gave positive tests for ammonia. Hairy root induced a slightly acid reaction and gave negative tests for ammonia.

The 3 amino acids were employed separately as a nitrogen source. A basic salt medium was prepared in 3 different lots: to the first, aspartic acid was added; to the second, glutamic acid; and to the third, tyrosine. Seeding and incubation temperatures were like those in previous experiments.

Hydrogen-ion concentrations were determined and qualitative nitrogen tests were made after a month. The culture media seeded, respectively, with both strains of crown gall and containing, respectively, aspartic and glutamic acids showed a yellow color, pellicle formation, and an alkaline reaction. Growth on basic salt media containing tyrosine showed neither color nor pellicle formation and had an acid reaction. The crown-gall cultures were not differentiated.

Ammonia was present in the inoculated culture of aspartic and glutamic acids. The cultures grown on tyrosine and the control tubes gave negative tests.

Carbon Metabolism

Studies of carbon metabolism (Pinckard, 5; Riker *et al.*, 8; Sagen *et al.*, 10) have been concerned with the utilization of various carbon sources at favorable temperatures. Certain of these investigations have been repeated with quantitative methods on cultures grown above and below the critical temperatures. Previous work concerned with the availability of various carbon sources has shown both glucose and sucrose to be good sources for crown gall, hairy root, and Radiobacter organisms. These sugars were employed in media with the following formula referred to as the basic salt media: glucose or sucrose, 5.0 g.; magnesium sulphate ($MgSO_4 \cdot 7H_2O$), 0.2 g.; sodium chloride (NaCl), 0.2 g.; calcium chloride ($CaCl_2$), 0.1 g.; dibasic potassium phosphate (K_2HPO_4), 0.2 g.; potassium nitrate (KNO_3), 5.0 g.; and distilled water to make 1000 cc.

The salts and sugar in neutral solution were autoclaved separately and then mixed and tubed aseptically, with exactly 10 cc. in each tube. The tubes were seeded as previously described. After 6 weeks' incubation at 28° and 32° C., respectively, quantitative sugar determinations were made according to the method of Stiles, Peterson, and Fred (12). The glucose sugar cultures were analyzed directly. The sucrose cultures were hydrolyzed according to standard procedure by heating with hydrochloric acid at 70° C. for 10 minutes. The pH readings were made with a glass electrode. The results are given in table 2.

TABLE 2.—*Fermentation of glucose and sucrose by P. tumefaciens and B. radiobacter at 28° and 32° C.*

Organism	Incubated at 28° C.		Incubated at 32° C.	
	pH	Sugar[a] fermented	pH	Sugar[a] fermented
		Per cent		*Per cent*
		Glucose		
Pathogenic *P. tumefaciens*	7.8	100	8.3	100
Attenuated *P. tumefaciens*	7.6	100	8.2	100
B. radiobacter	9.6	58	9.9	56
Control	7.4	0	7.4	0
		Sucrose		
Pathogenic *P. tumefaciens*	9.1	96	9.0	95
Attenuated *P. tumefaciens*	8.8	94	7.9	88
B. radiobacter	9.8	47	9.7	47
Control	7.7	0	7.7	0

[a] In each case the amount of sugar in the control tubes was taken as 100 per cent.

A study of the cultural characteristcis showed no differences between the pathogenic and attenuated crown-gall cultures, since both grew abundantly, forming pellicles in both sucrose and glucose. Radiobacter produced traces of pellicle formation which disappeared after a few days. There were no visible differences in the growth produced above and below the critical temperature range.

Sugar metabolism at 28° and 32° C. was very similar, both pathogenic and attenuated strains of crown gall fermenting all the glucose present; slightly less of the sucrose was fermented by these organisms. Radiobacter was found to utilize about one-half of the sugar present, using somewhat more glucose than sucrose. Hydrogen-ion determinations, too, were similar at both 28° and 32° C. The crown-gall cultures changed the reaction only slightly and the Radiobacter characteristically produced a very alkaline reaction in glucose. The reactions produced in sucrose were all basic. These studies indicate that the pathogenic and attenuated strains of crown gall are similar in their ability to ferment glucose and sucrose.

Two series of studies using ethyl alcohol as a carbon source were made, since its utilization differentiated crown-gall bacteria from *B. radiobacter* (Sagen *et al.*, 10). Basic nitrate medium was prepared as for the quantitative fermentation studies, except that the alcohol was substituted for sugar. The basic nitrate salts were autoclaved; the alcohol was sterilized by filtration and added. Sagen's results were confirmed regarding differences between pathogenic crown-gall bacteria and *B. radiobacter,* but no significant differences appeared between pathogenic and attenuated crown-gall cultures, whether below or above the critical 28° to 30° C. temperature range. Representative pH readings were as follows: pathogenic crown gall at 28° C., 4.9; at 32° C., 4.7; attenuated crown gall at 28° C., 4.9; and at 32° C., 4.6.

The production of carbon dioxide was measured during a single trial in sealed Eldrich tubes containing barium hydroxide on one side and seeded 1-per cent yeast infusion, 1-per cent glucose, and mineral salts medium on the other. The cultures used were pathogenic and attenuated crown-gall bacteria and hairy-root bacteria, respectively. Some tubes of each culture were incubated at 28° C. and others at 32° C. for 18 days at each temperature. The crown-gall cultures had changed the medium from pH 7.7 to 6.7–6.9 and the hairy-root cultures to 4.2–4.4 at both temperatures. The amount of carbon dioxide evolved by all cultures from 20 cc. of media varied from 0.024 to 0.027 g. More details on carbon dioxide production by these organisms are reported by Conner, Riker and Peterson (1). This evidence does not support the working hypothesis that carbon dioxide may be the stimulating material.

Hydrogen sulphide production was examined in a single trial with those same cultures at the two stated temperatures. A peptone-mineral salts medium was used. The cultures were fitted with lead acetate filter paper. All showed strongly positive tests.

SEROLOGICAL TESTS

Various immunological tests, particularly agglutination, have helped to differentiate closely related bacterial organisms. Since the specific organism is agglutinated by its homologous antisera at higher titers than even closely related organisms, 5 series of agglutination tests were run to determine the relationship of the pathogenic and attenuated strains of *Phytomonas tumefaciens.*

Rabbits were used for the production of antisera.

The antigens were prepared by suspending the growth of 48-hour cultures on yeast-water-potato-dextrose in 0.85-per cent salt solution. In 1 cc. of the suspension there were approximately 200,000,000 organisms, according to the Burroughs Wellcome Co. opacity tubes for standardization of vaccines.

In order to get the greatest possible distribution of organisms, both intravenous and intraperitoneal injections were made. Four injections were made at 4-day intervals. The doses were 0.5 cc., 1.0 cc., 3.0 cc., and 5.0 cc. For the third and fourth doses, 1- and 2-cc. portions of the total dose, respectively, were injected into the veins. After a rest period of 3 weeks, these animals were reinjected to increase the antibody titer. First 0.5 cc. was injected intraperitoneally, and 6 days later 1 cc. was injected intravenously.

Dilutions of normal sera and antisera were made with 0.425-per cent salt solution. Doubling dilutions of the normal sera starting from 1–5 to 1–1,280 and of the antisera from 1–20 to 1–10,240 were set up in serological tubes.

Bacterial suspensions in 0.425-per cent solutions were diluted so that, when 7 drops of the antigen were added to 1 cc. of salt solution, the suspension was slightly turbid. Readings were taken after the solution had stood 24 hours at room temperatures.

With the first series of rabbits, titers up to 1–5,120 and sometimes 1–10,240 were secured with homologous sera. The antiserum produced with virulent cultures (A6) agglutinated attenuated cultures (A6–6) only at 1 to 640 or less. Likewise, antiserum produced with attenuated cultures agglutinated virulent cultures only at 1 to 320 or less. Virulent cultures grown at 28° and 32° C. showed no difference in agglutination. Similar results were secured with attenuated cultures.

The second, third, and fourth series[3] of rabbits, however, failed to differentiate the virulent and attenuated cultures. In the last two series the usual agglutination trials were supplemented by agglutinin absorption tests. In no case was a differentiation secured between the virulent and attenuated cultures. In the last 2 series, cultures attenuated with glycine (13), also were included.

The details of these experiments have been omitted because of their volume and the similarity of results. These data present further evidence of the similarity in origin of these cultures. It appears that pathogenicity is independent of any factor associated with the agglutinins.

OSMOTIC PRESSURE

The importance of osmotic pressure has been emphasized in various early publications. Smith (11) considered that certain substances, namely, aldehydes, acetones, alcohols, acids, and alkalies, produced in the plant by the bacteria act together in producing tumors. This action he thought to be mainly osmotic rather than chemical, the osmotic pressure being raised by diffusion of metabolites into the cells. With this concept in mind, the pathogenic and attenuated crown-gall cultures were grown above and below the critical temperature range, not only in culture media, but also in a medium as similar to plant juices as possible. For this reason, tomato extract was chosen for preliminary trials and prepared in such a manner as to change it as little as possible. Freezing points were determined at intervals covering at least a month. The osmotic pressures were calculated from freezing points according to the table given by Harris and Gortner (2).

Tomato-extract media in Series 1 and 2 were prepared according to the same method. A dilution of one part of fresh tissue with 2 parts of water by weight was necessary because stronger extracts often are relatively toxic to bacteria. The tissue was finely cut up, placed in a suction flask, and

[3] Portions of these studies were made by J. M. Van Lanen and Margaret M. Mills.

kept in a water bath at 65° C. for 2 hours under the partial vacuum from a water pump. The juice extracted was filtered through filter paper, the volume made up to the original, and portions of 160 cc. each were placed in bottles, except in Series 2, where 40 cc. was placed in each bottle. In the second series, cotton plugs were used; in all other series caps were used. The bottles were autoclaved at 8 pounds' pressure for 75 minutes. The juice was extracted somewhat differently in Series 3. The tissues were frozen at approximately – 15° F. for 20 hours, steamed for 1 hour, and then autoclaved at 17 pounds for 1 hour.

For Series 4 and 5, yeast-water dextrose was used. It was prepared according to the following formula: yeast infusion (1 per cent), 500 cc.; sodium chloride ($NaCl$), 0.2 g.; magnesium sulphate ($MgSO_4$), 0.2 g.; calcium sulphate ($CaSO_4$), 0.1 g.; dextrose, 10 g.; 20-per cent phosphate solution adjusted to pH 6.87 (a mixture of K_2HPO_4 and NaH_2PO_4), 50 cc.; distilled water to make 1000 cc. 160-cc. amounts were placed in bottles and autoclaved at 15 pounds for 1 hour.

The inoculum in all cases was grown on yeast-water-mannitol agar slants (Hendrickson, *et al.*, 3). In the preliminary experiments only the pathogenic crown-gall cultures were used. These bottles were incubated at 24° C. In subsequent experiments the attenuated culture was also used. In all experiments, except the preliminary one, duplicate sets of cultures were incubated at 28° and 32° C. The inoculum of Series 1, 3, 4, and 5 consisted of 1 cc. of a suspension of the bacterial organisms equal in density to 0.8-per cent $BaSO_4$ (McFarland, 4). Since there was only 40 cc. of media in the bottles in Series 2, 0.2 cc. of inoculum was used. The bottles were incubated and freezing points determined on the day of inoculation and at intervals thereafter.

A Beckmann thermometer was used to determine the freezing points of the cultures and controls. The freezing mixture was prepared by adding $CaCl_2$ to chopped ice, and the temperature was kept between – 7° and – 10° C. At least 4 samples of 40 cc. each were run. The freezing point of water was determined by averaging at least 10 determinations. Using the difference between the freezing point of the media and water, the osmotic pressure was read directly from the Harris and Gortner (2) table. Hydrogen-ion concentrations were tested by the quinhydrone method each time freezing point determinations were made. In all but the preliminary series the amount of undercooling was considered.

No differences in osmotic pressure produced by pathogenic and attenuated crown-gall cultures, above and below the critical-temperature range, were found in Series 3. Variations in osmotic pressure from interval to interval occurred, attributable probably to the bacterial utilization of different fractions of the medium.

The two last series of freezing points were done on cultures of the yeast-water-dextrose medium. Although variations similar to those found in tomato extract were found during the early part of the incubation period, these two series both showed a decrease in osmotic pressure of cultures after about the 18th day. This was probably because the sugar was changed to carbon dioxide, or to bacterial gum having less osmotic activity. The results of Series 1, 2, and 3 are omitted because there was no significant change in osmotic pressure of any of the cultures. Those from Series 4 and 5 are averaged and given in figure 1.

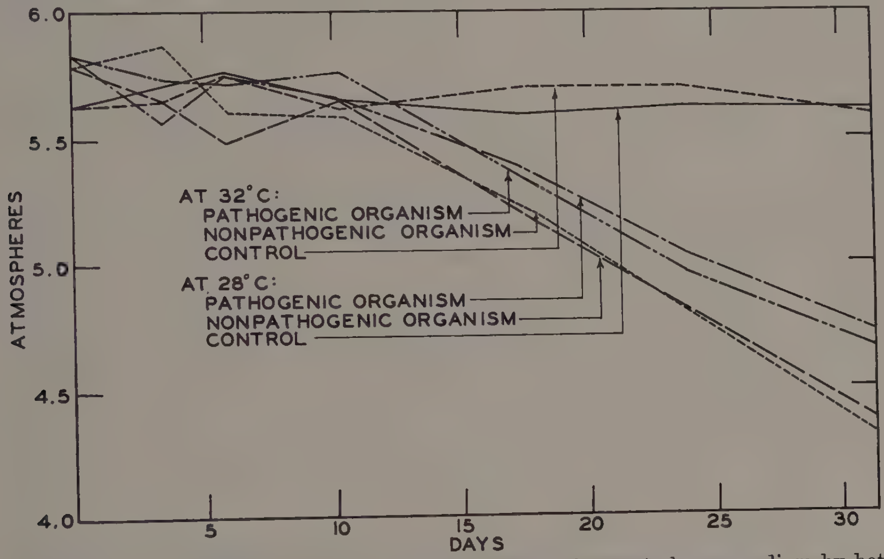

FIG. 1. The changes in osmotic pressures produced in yeast-glucose medium by both pathogenic and non-pathogenic cultures grown, respectively, at 28° and 32° C.

Hydrogen-ion concentrations recorded parallel with the freezing point readings showed no significant differences between the pathogenic and attenuated cultures. Those incubated above the critical temperature range were similar to those incubated at 28° C. Cultures grown on tomato extract showed that both the pathogenic and attenuated cultures produced an alkaline reaction of 8.0 or over. The controls became slightly more acid. The cultures grown in yeast-water-dextrose changed less than pH 0.16 hydrogen-ion concentration.

These results indicate that both the virulent and nonpathogenic cultures produce small variations but no striking change in tomato extracts. While fluctuations occurred, particularly in the first few days after seeding, these, so far as could be determined, were essentially similar for both cultures. When an abundance of sugar was present, both cultures lowered the osmotic pressure doubtless as a result of sugar utilization. Here again there was no significant difference between the cultures. If anything, the virulent culture produced less change than the attenuated culture. Likewise, cultivation above and below the critical temperatures showed no significant differences.

Measurements of the osmotic pressure in crown-gall and healthy tissue were attempted by two methods: (a) Sections of healthy and diseased tissue were placed, respectively, in sugar solutions of known and graded concentrations. The strength of the sugar solution which would produce plasmolysis was noted. (b) Small pieces of gall tissue and of healthy tissue, respectively, were punctured with a copper-iron thermocouple made from wire 0.1 mm. in diameter. The iron wire was joined to copper wire in the cold junction. The two copper leads were connected to a high sensitivity galvanometer having copper terminals. The galvanometer had been standardized against the freezing point of water and that of graded sugar solutions.

The results from both methods showed considerable variability. Different parts of the same gall and likewise different portions of the same stem, depending on the tissue involved, gave unlike determinations. For this reason the detailed results are omitted. In general, however, the gall had an osmotic pressure similar to embryonic tissue, both being somewhat higher than that of uninoculated stem tissue.

In view of these results it seems necessary to reserve judgment about osmotic pressure being the chief factor in the production of crown gall. However, it seems likely that the bacteria may produce small localized changes, as a result of their metabolism in the tissue, that may be important in the complex.

HOST-PARASITE RELATIONS

Growth of Bacteria in the Host

Since the attenuated culture of crown gall produces no galls and the pathogenic culture fails to produce galls on tomato plants at 32° C. and above, a study was undertaken of the rate of multiplication of the bacteria under these conditions in the host. The aim was to determine whether the failure to produce galls was because of a bacteriostatic effect of the host tissue. Accordingly, several series of plant inoculations have been set up and dilution plate counts made at intervals with the inoculated tissue.

The methods used in these series of experiments were the same, except for variation in the number of bacteria introduced. The cultures employed were grown 48 hours on slants of yeast-water-mannitol agar. The suspensions were made in sterile lake water and were approximately equal in density to the 1-per cent barium sulphate standard (McFarland, 4). In all except Series 4, these suspensions were diluted 1–100 before inoculations were made. In Series 4 suspensions equal in density to this 1-per cent standard were used for inoculation.

The inoculation each plant received was approximately .02 cc. of the suspension. It was introduced by a standardized capillary tube. The number of bacteria introduced was estimated by pouring plates of the original material.

After inoculation, the wounded portion of the plant was wrapped with a narrow strip of adhesive tape to prevent the tissue from drying out. There was no disinfectant in this tape. The plants were placed, respectively, in chambers at 25° to 28° C. (below the critical temperature range) and at 31°

and 34° C. (above the critical temperature range). Each chamber was illuminated for 12 hours daily with six 500-watt lamps cooled by running water.

The number of bacteria in each plant inoculation was determined by plate counts on yeast-water-mannitol agar. These were made immediately after inoculation and at subsequent intervals thereafter. In Series 1, 2, and 3, tissue was taken from duplicate plants. In Series 4 and 5, the plants were inoculated in triplicate.

The method for determining the number of bacteria present was as follows: A section of stem 1 cm. above and 1 cm. below the point of inoculation was removed and macerated as completely as possible with sterile sand and 10 cc. of sterile lake water in a sterile mortar. Further dilutions were made in bottles and tubes according to common procedure. The counts were made after 3 or 4 days' incubation at room temperature on plates that almost always contained between 50 and 500 colonies.

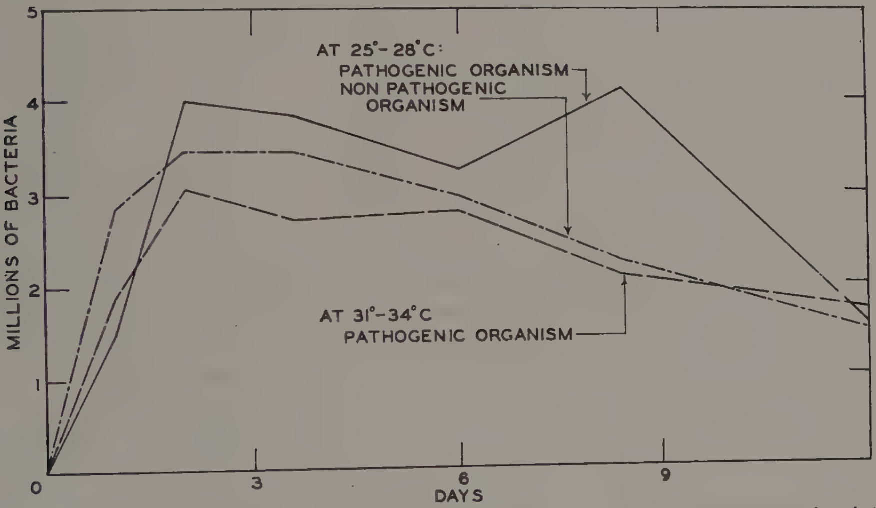

FIG. 2. Growth of virulent and attenuated crown-gall bacteria after inoculation into tomato stems growing below (25°–28° C.) and above (31°–34° C.) the critical temperatures for gall formation on tomato.

Four preliminary experiments were carried out during the development and improvement of the technique. Although the results are omitted, they are in general agreement with those reported. Five series of trials are summarized in figure 2. The details of one representative trial are given in table 3.

Before interpreting these results one may recall that the bacteria are introduced into wounds. The contents from ruptured cells serve, both in the injured cells and the fluid which passed into the intercellular spaces, as a cultural medium for the bacteria. Viewed in this regard there are obvious variations depending upon the size of the wound, the tissue involved, and the volume and distribution of the extravasated cell contents. Thus variations may appear in the growth curves of the bacteria.

TABLE 3.—*Change in number of virulent and attenuated crown-gall bacteria after inoculation into tomato plants*

Days after inoculation	Plant no.	Bacterial plate counts—000 omitted			
		Virulent culture		Attenuated culture	
		Plants at about 27° C.	Plants at about 32° C.	Plants at about 27° C.	Plants at about 32° C.
		No.	*No.*	*No.*	*No.*
0	1	6.7	6.7	6.7	6.7
	2	7.3	7.3	5.6	5.6
	3	11.3	11.3	7.1	7.1
1	1	900	1400	530	470
	2	700	290	530	1190
	3	340	590	1140	730
2	1	1300	620	1300	1690
	2	1580	770	1890	1800
	3	3180	1290	1140	1300
4	1	1120	1680	1660	1310
	2	1760	850	1480	1890
	3	....	820	4500	1910
8	1	2210	460	2130	780
	2	4030	920	1260	680
	3	2720	830	1480	2040
12	1	532	230	202	366
	2	452	216	347	282
	3	239	221	390	127

In relation to bacterial counts, the distribution of the bacteria as individuals, or as small or large clumps, is influenced by various factors. These include the compactness of the bacterial mass, the density of the gum which embeds them, and the electric charge they carry. Likewise, the number of loci, (containing one or more bacterial cells) which produce colonies as discussed by Riker and Baldwin (7), is influenced by such factors as the vigor and distribution of the bacteria and the suitability of the medium. It is common for direct counts to be 10 to 100 times as large as plate counts. When these factors are considered, the variations are not surprising. Relative uniformity has frequently occurred in the first day or two. It is apparent from the curves in figure 4 that the host plant has no significant bacteriostic effect upon either the virulent or the attenuated crown-gall organism at either of the temperatures employed. Thus, neither the failure of gall development in tomato at 31° C. nor that following inoculation with the attenuated culture can be attributed to a deficiency in bacterial growth within the plant.

In 4 of the 5 series a secondary rise in the pathogenic organism occurred after about 7 or 8 days. The first visible signs of gall formation were noted on about the sixth day. While no experimental evidence is available to explain this secondary rise, it seems possible that the swelling of cells under the pressure of the bacteria may reach a point at this time when the pressure about some of these swollen cells may burst a few of them. Such collapse has been observed frequently in gall tissue (*e.g.,* Riker, 6). Thus, with an additional amount of medium available for bacterial growth the number may increase.

Since the tomato has no bacteriostatic effect on the cultures above and below the critical temperature, the question remains whether this critical temperature has its effects owing to a change in the bacteria or a change in the host.

Host Temperature Studies

The production of galls at 28° C. and below and the failure of gall production at 32° C. and above in tomato plants indicate that temperature is correlated with the physiological activity involved in pathogenicity. Since only the tomato plants have been used, other hosts were included to determine whether this physiological activity concerned the bacteria, the tomato host, or other hosts. The plants selected for further tests along with tomato were

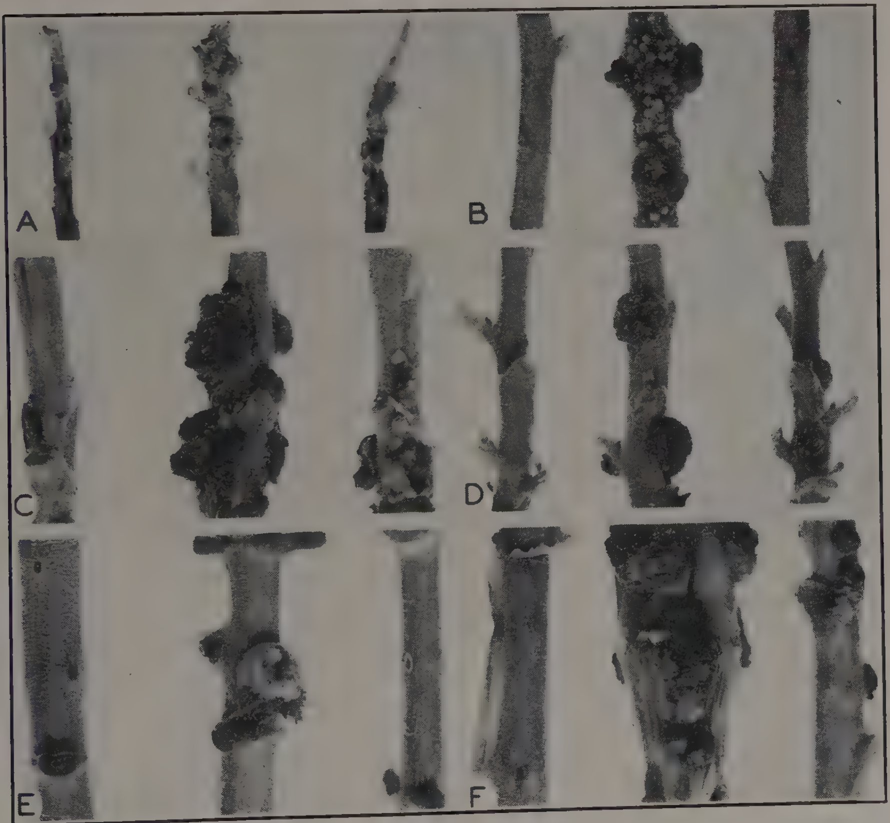

FIG. 3. Response of various plants to crown-gall inoculations at various temperatures. A. *Sedum spectabile,* 5 weeks after inoculation; B. *Lycopersicum esculentum,* 4 weeks; C. *Nicotiana glutinosa,* 4 weeks; D. *Nicotiana tabacum,* 4 weeks; E. *Bryophyllum calycinum,* 5 weeks; and F. Hybrid of *N. glutinosa* and *N. tabacum,* 4 weeks. In every case stems having punctures only are at the left, inoculated stems grown at approximately 27° C. are in the center, and inoculated stems grown at approximately 31° C. are at the right.

Sedum, Bryophyllum, Nicotiana glutinosa, Nicotiana tabacum, and a cross between *Nicotiana tabacum* and *Nicotiana glutinosa.*

The treatment of the plants involved puncture inoculation of the young stems with a dissecting needle carrying virulent bacteria. Four plants of each kind were thus inoculated. Controls were merely punctured. Duplicate sets were placed in the chambers previously mentioned that were operating at approximately 27° C. and 31° C., respectively. All of the plants that were merely punctured were grown at the higher temperature.

Some plants reacted like the tomato plant and others formed galls above the critical temperature for tomato plants. *Sedum* and *Bryophyllum* were found to produce gall, like tomato, only at 28° and below, whereas all of the species of *Nicotiana* used produced galls both above and below the critical range. However, the galls produced above the critical temperature range were considerably smaller and made their appearance from a week to 10 days later than those produced at 28° C. and below (Fig. 3). The punctured controls were negative in all cases. Only 1 series of *Bryophyllum* has been studied, but at least 2 series of each of the other species have been observed. Because these plants made satisfactory growth at the higher temperatures, the failure in gall production seems not to be attributable to lack of plant growth. Since the bacteria induce galls on some plants at the higher temperature, the effect temperature has on pathogenicity seems correlated more with the host than with the bacteria.

SUMMARY

Crown-gall, attenuated crown-gall, Radiobacter, and hairy-root bacteria have been studied comparatively in relation to various physiological characters. The virulent crown-gall cultures were carried both above 28–30° C., where no galls develop on tomato, and below this temperature, where they develop well.

The crown-gall, attenuated crown-gall, and Radiobacter bacteria were similar in the utilization of various sources of nitrogen, various sources of carbon, and in the production of carbon dioxide and hydrogen sulphide. The virulent bacterial cultures, incubated both above and below the critical temperature for pathogenicity, were likewise similar in these characters. The hairy-root bacteria failed to initiate growth upon inorganic sources of nitrogen. It produced acid from certain sugars, unlike the crown-gall bacteria.

The virulent and attenuated crown-gall cultures appeared similar in agglutination and agglutinin-absorption tests. Cultures grown both above and below the critical temperatures showed no significant differences. These serological studies suggest that pathogenicity is independent of any factor associated with agglutinin.

The virulent and attenuated crown-gall bacteria were similar in producing minor fluctuations in the osmotic pressure of tomato extract. In a sugar medium both lowered the osmotic pressure. Cultures above and below the critical temperatures were similar.

Both the virulent and attenuated bacteria grew well in the host tissue on both sides of the critical temperature.

No galls developed on tomato, *Sedum,* and *Bryophyllum* above the critical temperature. However, galls were produced above this point on several kinds of tobacco.

LITERATURE CITED

(1) CONNER, H. A., A. J. RIKER, and W. H. PETERSON. The carbon metabolism of the crown-gall and hairy-root organisms. Jour. Bact. **34**: 221–236. 1937.

(2) HARRIS, J. A., and R. A. GORTNER. Notes on the calculation of the osmotic pressure of expressed vegetable saps from the depression of the freezing point, with a table for the values of P for Delta = 0.001° to Delta = 2.999°. Amer. Jour. Bot. **1**: 75–78. 1914.

(3) HENDRICKSON, A. A., I. L. BALDWIN, and A. J. RIKER. Studies on certain physiological characters of *Phytomonas tumefaciens, Phytomonas rhizogenes* and *Bacillus radiobacter.* Part II. Jour. Bact. **28**: 597–618. 1934.

(4) MCFARLAND, J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. Jour. Amer. Med. Assoc. **49**: 1176–1178. 1907.

(5) PINCKARD, J. A. Physiological studies of several pathogenic bacteria that induce cell stimulation in plants. Jour. Agr. Res. [U. S.] **50**: 933–952. 1935.

(6) RIKER, A. J. Some morphological responses of the host tissue to the crowngall organism. Jour. Agr. Res. [U. S.] **26**: 425–436. 1923.

(7) ————, and I. L. BALDWIN. The efficiency of the poured-plate technique as applied to studying bacterial plant pathogens. Phytopath. **29**: 852–863. 1939.

(8) ————, W. M. BANFIELD, W. H. WRIGHT, G. W. KEITT, and H. E. SAGEN. Studies on infectious hairy-root of nursery apple trees. Jour. Agr. Res. [U. S.] **41**: 507–540. 1930.

(9) ————, and T. O. BERGE. Atypical and pathological multiplication of cells approached through studies on crown gall. Amer. Jour. Cancer **25**: 310–357. 1935.

(10) SAGEN, H. E., A. J. RIKER, and I. L. BALDWIN. Studies on certain physiological characters of *Phytomonas tumefaciens, Phytomonas rhizogenes,* and *Bacillus radiobacter.* Part I. Jour. Bact. **28**: 571–595. 1934.

(11) SMITH, E. F. Mechanism of tumor growth in crowngall. Jour. Agr. Res. [U. S.] **8**: 165–188. 1917.

(12) STILES, H. R., W. H. PETERSON, and E. B. FRED. A rapid method for the determination of sugar in bacteriological cultures. Jour. Bact. **12**: 427–439. 1926.

(13) VAN LANEN, J. M., I. L. BALDWIN, and A. J. RIKER. Attenuation of cell stimulating bacteria by specific amino acids. Science **92**: 512–513. 1940.

(14) WRIGHT, W. H., A. A. HENDRICKSON, and A. J. RIKER. Studies on the progeny of single-cell isolations from the hairy-root and crown-gall organisms. Jour. Agr. Res. [U. S.] **41**: 541–547. 1930.

SYNTHESIS OF TOBACCO MOSAIC VIRUS PROTEIN IN RELATION TO LEAF CHROMOPROTEIN AND CELL METABOLISM[1]

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INTRODUCTION

Since infection with tobacco mosaic virus (*Marmor tabaci* Holmes) results in chloroplast derangement in certain suscept species (16, 17, 5, 13, 4, and others) a possible connection between the virus protein and the chlorophyll protein was suggested. Recent work of Smith and Pickels (12), and results obtained in our own laboratory, have indicated that not only the chlorophylls but also the carotins and xanthophylls are chemically linked to protein.

Woods (16) was the first to indicate the role of oxidizing enzyme systems in the destruction of chlorophyll in virus-infected plants. Peterson and McKinney (10) have shown a correlation between destruction of the plastid pigments and increases in chlorophyllase activity in mosaic-infected tobacco. All the pigments were affected proportionately. Sorokin (13) and Dufrenoy and Dufrenoy (5) have indicated that an actual proteolytic disintegration of the chloroplast occurs as a result of infection with tobacco-mosaic virus. So far as we are aware no one has presented data dealing quantitatively with the effect of a virus on the chromoproteins *per se,* although Loring *et al.* (8) and Price and Wyckoff (11) have reported isolating chlorophyll-green proteins with half the sedimentation constant of tobacco-mosaic virus, from healthy plants in the ultra-centrifuge. Price and Wyckoff (11, p. 93) state "It would seem to be of fundamental importance to determine how closely the macromolecules are associated with the chlorophyll in living cells." They also suggest that these "macro-molecular substances" might be related to virus protein.

Anson (1) has recently called attention to several methods of extracting chlorophyll proteins. We found these methods unsuited to the extraction of tobacco chromoproteins because of the rapid alteration of the proteins due to oxidative action. Furthermore, a method was required that would allow quantitative separation of the virus protein from the chromoprotein fraction. Some investigators in the preparation of virus-infected material have resorted to freezing. Freezing, as demonstrated by Anson (1), denatures the chromoprotein. Our own results also show that, following the usual procedures, excessive oxidation occurs (phenol-dehydrogenase systems in particular), which gives dark-brown preparations of the virus and a complex of altered proteins. Methods were developed that allowed a quantitative separation to be made between the chromoprotein complex and virus protein avoiding denaturation. Since little has been published as to the nature of tobacco chromoprotein ("chlorophyll-protein") certain characteristics of this substance were also determined from specially purified preparations. It has been possible to develop some concepts as to the inter-relation of nitrogen metabolism, including chromoprotein and virus synthesis, and specific respiratory activity of the cell.

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[1] Expense of publication of this paper was borne by its authors.

[2] Scientific Paper A8. Contribution No. 1745 of the Maryland Agricultural Experiment Station (Department of Botany).

EXPERIMENTAL METHOD

Plants of *Nicotiana tabacum* var. Turkish, or hybrids involving *N. glutinosa,* were used in all experiments. They were generally grown in coarse quartz sand and watered once a day with nutrient solutions. In controlled nitrogen-level experiments the following solution was employed:

$Ca(NO_3)_2 \cdot 4H_2O$	0.005	Molar (or varied)
$MgSO_4 \cdot 7H_2O$	0.002	Molar
KH_2PO_4	0.0016	Molar

One (1) cc. of 0.1% Ferric tartrate per liter.

A composite solution containing traces of $MnSO_4$, $ZnSO_4$, $CuSO_4$ and H_3BO_3 was added. For the minus nitrogen solution $CaCl_2$ was substituted for $Ca(NO_3)_2$.

A green-mottling single-lesion strain of common tobacco mosaic virus (*Marmor tabaci* Holmes), or a "yellow" derivative was used in all infection experiments. Inoculation was accomplished by rubbing the half leaf in the usual way with cheesecloth-covered spatulas, previously dipped in freshly extracted infectious juice. One half of each experimental leaf was inoculated, the other half serving as a control. In certain experiments the terminal bud and all axillary buds were removed prior to inoculation to prevent complications arising from translocation of nitrogenous substances to the meristematic zones. Respiratory activity was measured in terms of protoplasmic streaming, as previously described (19, 20).

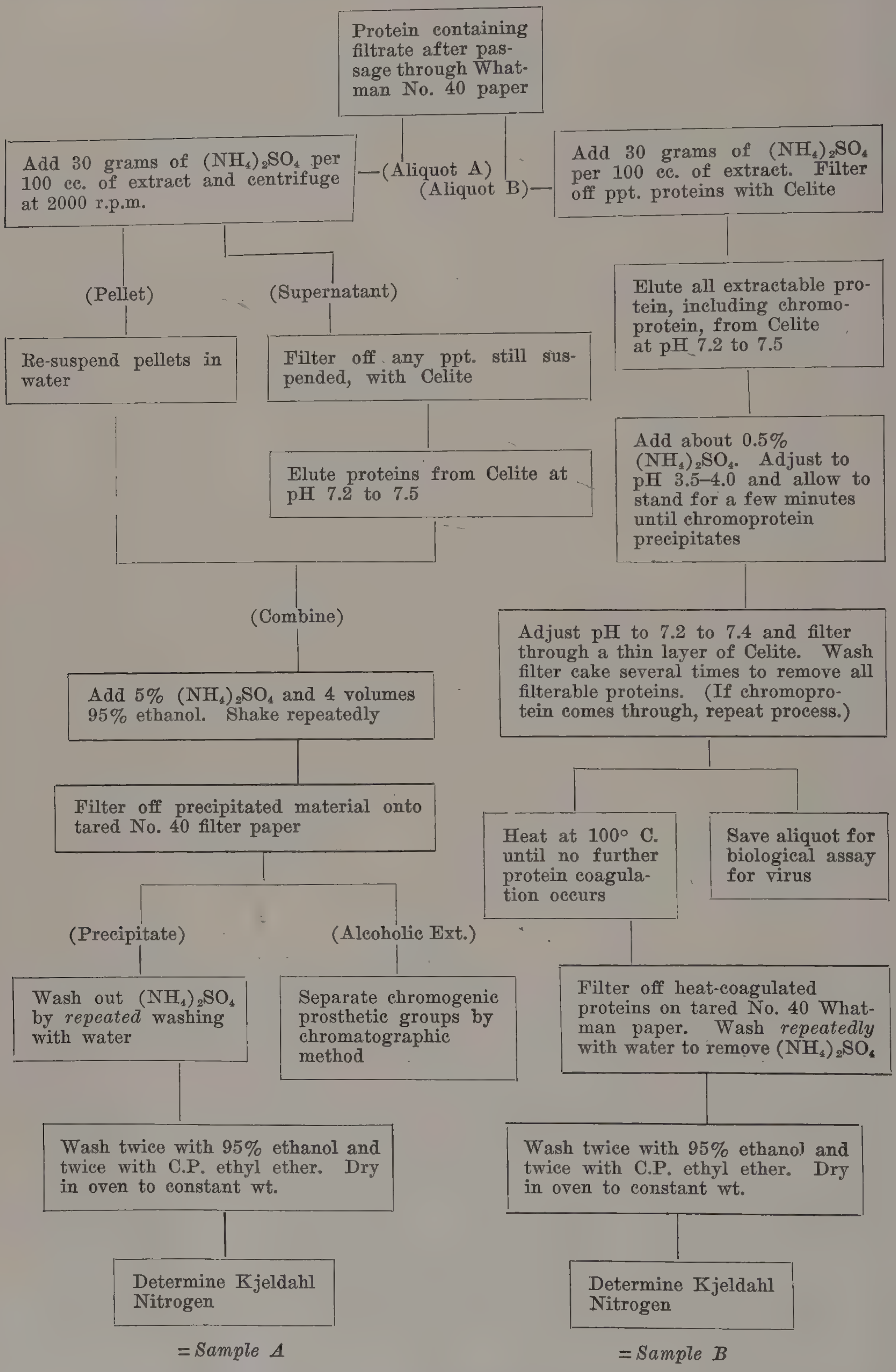
The following procedure gave the most highly purified and stable preparations of chromoprotein.

(a) 25 to 50 g. of fresh leaf tissue (mid-ribs excluded) were thoroughly ground with quartz sand in a mortar containing about 100 cc. of a 0.5 per cent ammonium sulphide solution (temperature 5 to 10° C.). The liquid-extracted material was squeezed out through cheesecloth and the residue re-extracted until nearly colorless.

(b) The combined extract was filtered with suction through coarse filter paper and finally through Whatman No. 40 filter papers. 30 g. of ammonium sulphate was added to each 100 cc. of filtrate and the precipitated proteins filtered off with Celite.

(c) The filter cake was washed several times with fresh 30 per cent ammonium sulphate, and finally with progressively more dilute ammonium sulphate solutions until the chromoproteins just started to come through. The non-colored protein fractions removed by the dilute ammonium sulphate solutions were discarded.

TABLE 1. *Procedure for quantitative determination of chromoprotein, tobacco-mosaic virus protein, and certain other proteins in Turkish tobacco. The proteins are extracted from the tissues as described under experimental method, item (a) of the qualitative procedure*


Protein containing filtrate after passage through Whatman No. 40 paper
—(Aliquot A)
(Aliquot B)—
Add 30 grams of $(NH_4)_2SO_4$ per 100 cc. of extract and centrifuge at 2000 r.p.m.
Add 30 grams of $(NH_4)_2SO_4$ per 100 cc. of extract. Filter off ppt. proteins with Celite
(Pellet)
(Supernatant)
Re-suspend pellets in water
Filter off any ppt. still suspended, with Celite
Elute proteins from Celite at pH 7.2 to 7.5
Elute all extractable protein, including chromoprotein, from Celite at pH 7.2 to 7.5
Add about 0.5% $(NH_4)_2SO_4$. Adjust to pH 3.5–4.0 and allow to stand for a few minutes until chromoprotein precipitates
(Combine)
Add 5% $(NH_4)_2SO_4$ and 4 volumes 95% ethanol. Shake repeatedly
Adjust pH to 7.2 to 7.4 and filter through a thin layer of Celite. Wash filter cake several times to remove all filterable proteins. (If chromoprotein comes through, repeat process.)
Filter off precipitated material onto tared No. 40 filter paper
Heat at 100° C. until no further protein coagulation occurs
Save aliquot for biological assay for virus
(Precipitate)
(Alcoholic Ext.)
Wash out $(NH_4)_2SO_4$ by *repeated* washing with water
Separate chromogenic prosthetic groups by chromatographic method
Filter off heat-coagulated proteins on tared No. 40 Whatman paper. Wash *repeatedly* with water to remove $(NH_4)_2SO_4$
Wash twice with 95% ethanol and twice with C.P. ethyl ether. Dry in oven to constant wt.
Wash twice with 95% ethanol and twice with C.P. ethyl ether. Dry in oven to constant wt.
Determine Kjeldahl Nitrogen
Determine Kjeldahl Nitrogen
= *Sample A*
= *Sample B*

1. *Protein sample A–Sample B = Chromoprotein Complex*
2. Sample B = Protein Fraction "P." (In diseased tissue contains the virus protein and certain nuclear and cytoplasmic proteins that also occur in the healthy leaf halves.)

(d) A considerable amount of chromoprotein could then be extracted by washing the filter cake with small quantities of distilled water. While the chromoprotein obtained in this way can be further purified by repeated precipitation with ammonium sulphate and elution, as just described, considerable quantities of the protein will be lost through adsorption to the Celite.

(e) The chromoprotein was finally dialyzed in cellophane tubes against tap water (24 hours) and distilled water (24 hours).

For a *quantitative* comparison of chromoprotein and virus protein in infected and healthy tissues, the procedure outlined in table 1 was found practicable. In the quantitative procedure the initial extraction of the proteins is carried out as described in the isolation of more highly purified preparations. The method is sufficiently rapid to allow multiple extractions from plants grown under various experimental treatments. It is important to work at a low temperature (5° to 10° C.) particularly in the stages prior to ammonium sulphate precipitation.

EXPERIMENTAL RESULTS

Effect of Nitrogen Starvation on Respiration

Since the multiplication of tobacco-mosaic protein is dependent on cyanide-sensitive respiratory enzyme systems of the cell ("A-system") (19, 20), it was thought important to determine what effect nitrogen-starvation might have on these respiratory processes. Preliminary observations had indicated that under conditions of nitrogen-starvation a reduction in activity occurred in the particular enzyme system necessary for virus multiplication. Measurements of cellular respiration in terms of protoplasmic streaming (see 19 and 20 for technique) show clearly that a sufficiently prolonged reduction in nitrogen supply brings about a marked decrease in activity of the "A-system" respiration. This system is probably in part identical with the cytochrome oxidase system of the cell (20). Table 2 illustrates the results of 3 typical series of measurements with leaves from plants starved for nitrogen 8 to 9 days, and leaves from plants supplied with 450 p.p.m. of nitrogen during the same period. In this case 6 Turkish tobacco plants about 40 cm. tall that had been grown with a nutrient solution containing about 100 p.p.m. of nitrogen were used. Measurements of protoplasmic streaming in plants grown under similar conditions showed that about 70 per cent of the respiration of the leaves was cyanide-sensitive. The plants were divided into 2 lots, and the sand about the roots thoroughly flushed with tap water. One lot of 3 plants was then watered daily with the nutrient solution containing no nitrogen and the second group was supplied daily with a solution containing 450 p.p.m. of nitrogen. Terminal buds were removed at the start of the experiment and the lateral buds pinched off, as they developed. Eight days after placing the plants on the two different nitrogen levels the minus nitrogen plants showed distinct nitro-

TABLE 2. *Effect of nitrogen starvation on cyanide-sensitive protoplasmic streaming in Turkish tobacco. Minus-nitrogen plants had been starved for nitrogen for 8 days. 450 p.p.m. of nitrogen were supplied to the plus-nitrogen plants*

Samples	Nitrogen supply	Rates of cyclosis in microns per second[a]		Condition of leaf
		Before KCN–treatment	After KCN–treatment	
1	−N	9.16 ± 0.55[c]	4.97 ± 0.40	Yellowish-green, but green dominant
	+N	11.80 ± 0.80	2.68 ± 0.04	Dark-green
2	−N	8.28 ± 0.43[d]	5.03 ± 0.30	Similar to Expt. 1
	+N	11.24 ± 0.48	4.42 ± 0.69	Similar to Expt. 1
3[b]	−N	7.70 ± 0.24	4.87 ± 0.32	Yellowish-green, but more yellow than in Expts. 1 and 2
	+N	10.40 ± 0.67	2.46 ± 0.26	Dark-green
Av.	−N	8.51	4.95	
	+N	11.14	3.18	

[a] Rate of cyclosis is an index to respiratory activity. Each value is the average rate of five representative cells.

[b] Measured nine days after beginning of nitrogen starvation.

[c] Standard error $= \sqrt{\frac{\Sigma a^2}{n(n-1)}}$

[d] Four cells measured.

gen deficiency symptoms. Protoplasmic streaming was measured in the marginal epidermal cells of leaves from each of the 6 plants. The data of table 2 show clearly that in the nitrogen-starved plants the cyanide-sensitive respiration, as indicated by protoplasmic streaming, constitutes a much smaller percentage of the total respiration than in the high nitrogen plants. This reduction is largely in the "A-system" respiration (20). It is interesting to note that, whereas nitrogen starvation brought about a decrease in activity of the "A-system," an increase in the cyanide-resistant system ("C-system") occurred. The multiplication of tobacco mosaic virus brings about a somewhat similar inversion in the "A" and "C" systems (19, 20). Other experiments have given essentially the same results, although extreme nitrogen starvation, until practically all of the chlorophyll is gone, sometimes brings about an almost complete loss in "A-system" activity. Observations show that in plants grown at levels of nitrogen below 100 parts per million the respiration of dark-green leaves is less resistant to cyanide than the respiration of light-green leaves. While this is partly a matter of "physiological age" nitrogen supply seems to be one of the important factors.

Respiration and Virus Synthesis

Since we have previously shown (18, 20) that virus synthesis is dependent on the activity of cyanide-sensitive respiratory enzymes, and that this system in its turn is dependent on nitrogen supply, it follows that a sufficiently prolonged deficiency of nitrogen would decrease the synthesis of virus by reducing the activity of the necessary enzyme system. That such is the case is shown by figure 2. Details will be discussed in relation to chromoprotein synthesis. A supply of *inorganic* nitrogen in itself, however, is not requisite to virus multiplication. A simple experiment serves to demonstrate these points. A full-grown leaf from a necrotizing hybrid (*N. tabacum* × *N. glutinosa*), grown at a medium nitrogen level, was removed from the plant and cut into 6 pieces. Each of 5 pieces was treated with HCN (tension over a 5 per cent solution of NaCN at 25° C.) for varying lengths of time before inoculation. Preliminary experiments demonstrated that the *uptake* of virus at the instant of inoculation is not prevented by cyanide (Table 3), although the reduction in number of lesions that may

Table 3. *Effect of HCN treatments before and after inoculation of F_1 hybrid N. tabacum × N. glutinosa. In this experiment three leaves were each divided into three sectors and the sectors treated as indicated. All HCN exposures were for one hour at the gas tension over 10 per cent NaCN at 23° C.*

Treatment of sectors	Av. number lesions per sq. in.	Total number lesions counted	Remarks
Check	8.73	192	First lesions distinct in 48 hours
Cyanide before inoculation	3.96	91	Lesion development delayed several hours
Cyanide after inoculation	4.20	108	Lesion development delayed several hours

Table 4. *The influence of HCN on lesion development in five treated sectors of a leaf of the F_1 hybrid N. tabacum × N. glutinosa. Exposures were to the tension of HCN over 5 per cent NaCN at 25° C. for time periods indicated*

Period of exposure to HCN (minutes)	Number of lesions developed				Remarks
	48 hr. after inoculation		72 hr. after inoculation		
	Av. per sq. inch	Total per sector	Av. per sq. inch	Total per sector	
None	37.5	219	Same?	Same? (coalesced)	Too much coalescence after 72 hr. to count
15	23.4	164	Same	Same	Slight retardation
30	15.6	83	21.3	114	More retardation than following 15-minute treatment
45	8.0	41	9.8	50	Much retarded
60	1.5	10	3.0	20	Very much retarded, still very small 72 hr. after inoc.
75	0.6?	4?	0.8	5	Very much retarded, still very small after 72 hr.

occur appears to be due to a blocking of virus multiplication in the originally wounded and inoculated cell until a variable "incubation period" is passed, *i.e.*, until the originally inoculated cell dies and is no longer able to transmit virus to adjacent cells. Treatments with gaseous HCN inhibit the "A-system" respiration for varying lengths of time, depending on the amount of HCN taken up by the tissues and the time required for this to be lost by diffusion subsequent to treatment. Table 4 indicates the degree of retardation in the lesions that became established and the reduction in amount of infection due to blocking of virus multiplication during the critical "incubation period." Figure 1, A, illustrates the results of a similar experiment in which one sector was rendered lesion-free by cyanide treatment. It is clear from such experiments that the decrease in number of lesions is directly proportional to the intensity of HCN effect, as is the retardation in development of symptoms. That cyanide treatment does not permanently alter the susceptibility of the leaf is proved by the fact that reinoculation of HCN-treated leaves results in formation of new lesions. For example, the non-lesioned sector shown in figure 1, A, was held in water-saturated air for 94 hours after cyanide treatment, during which time no lesions developed. Certainly all free nitrate was reduced by the leaf during this time. One-half of this sector was then reinoculated and a large number of lesions developed (Fig. 1, B). This proves that the cyanide effect is reversible and that virus can be synthesized in leaves that have been removed from all external sources of nitrogen for long periods of time.

Nitrogen Supply and Virus Synthesis

The amounts of virus protein formed in detached leaves of Turkish tobacco are likewise greater than would be expected on the basis of any small amounts of inorganic nitrogen that might conceivably remain in them. In one experiment one-half of each of 5 leaves was inoculated with the mosaic virus and the leaves immediately placed in a water-saturated atmosphere in the dark at approximately 25° C. Five days later there was obtained from the inoculated halves of the leaves, 117 mg. of virus protein per 100 g. of fresh tissue. Other similar experiments with detached leaves yielded from 85 to 196 mg. of virus protein per 100 g. of fresh leaf. These amounts of virus protein, on the basis of a 15 per cent nitrogen content, correspond to 12.75 and 29.4 mg. of atomic nitrogen per 100 g. of fresh tissue, respectively. Since it is known that nitrates are reduced almost instantly in green leaves, it seems improbable that this much nitrogen could come from stored nitrates.

Relation between Chromoprotein and Virus Protein

Since the previous data indicated a relationship between enzyme activity and virus multiplication, it became of particular interest to determine the substrate for virus synthesis. Because of the known effects of the mosaic virus on plastid proteins (13, 5), it was thought that chromoprotein

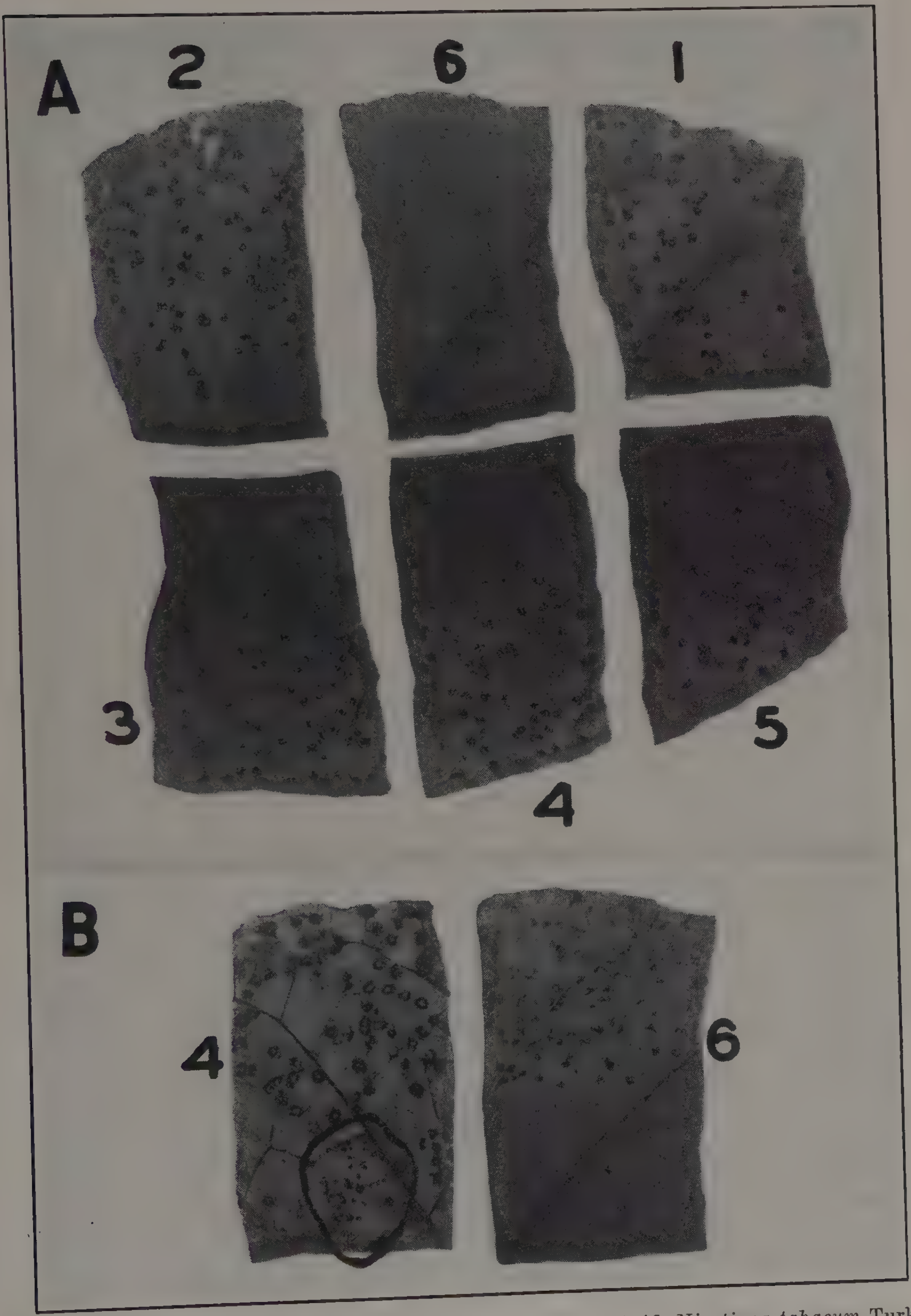


FIG. 1. Influence of HCN on lesion development in hybrid, *Nicotiana tabacum* Turkish × *N. glutinosa.* A. Six sectors, 4 days after inoculation: 1, Control; 2, treated 15 minutes; 3, treated 30 minutes; 4, treated 45 minutes; 5, treated 60 minutes; 6, treated 75 minutes. All treatments with HCN vapor over 5 per cent NaCN at 25° C. before inoculation. B. Sector 6, 8 days after start of experiment and 4 days after reinoculation of one-half of the sector. Sector 4, reinoculated at same time (encircled area).

("chlorophyll-protein") or its precursor might constitute this substrate in virus-infected cells. Little has been published concerning the nature of tobacco chromoprotein. The procedure outlined under "EXPERIMENTAL METHOD" for qualitative extraction of chromoprotein yielded preparations having the following properties:

1. Dialyzed preparations are stable with respect to dispersion. Samples have remained dispersed for 5½ months when kept quiescent in the refrigerator at 5° C.
2. Dialyzed chromoprotein is coagulated by prolonged heating at 100° C. In the presence of a trace of salt heat coagulation is speeded up.
3. In the presence of a trace of NaCl complete, reversible precipitation occurs between pH 4.9 to 5.1. Precipitation between 3.5 to 4.5 results in considerable aggregation and the formation of not readily redispersible precipitates.
4. The protein is irreversibly coagulated by freezing.
5. The protein is coagulated and prosthetic groups are removed by ethyl alcohol, methyl alcohol, acetone, and ethyl ether. A trace of salt facilitates the removal of the prosthetic groups by these reagents.
6. The white protein residue from ether precipitation gives positive biuret, xanthoproteic, and Millon reactions.
7. Digestion of the chromoprotein with 0.1 per cent commercial trypsin at 35° C. at pH 7.5 for 48 hours results in considerable breakdown in the protein with freeing of prosthetic groups.
8. Analysis of the prosthetic groups by the chromatographic method shows that chlorophylls A and B, in addition to carotins and xanthophylls, are present. These appear to be bound on the same protein, as evidenced by identical behavior during precipitation by various means.
9. The nitrogen content of the residue left after removal of the prosthetic groups with alcohol is between 14 and 15 per cent.

In order to determine the effect of tobacco-mosaic virus on the chromoprotein content of infected leaves, quantitative measurements (Table 1) were made of protein fractions from diseased and virus-free half leaves of plants that were grown under varying conditions of nitrogen supply. A number of experiments gave the same type of data. The results of a typical experiment are presented in figure 2. The 9 plants used in this experiment were grown on a medium nitrogen solution until they were about 40 cm. tall. They were then de-budded and supplied with the various levels of nitrogen. Five days later, when the minus-nitrogen plants showed appreciable symptoms of nitrogen deficiency, one-half of each leaf on plants in all series was inoculated with tobacco-mosaic virus. Approximately seven days after inoculation all of the plants were placed in total darkness for 24 hours at a temperature of approximately 25° C. to remove as much starch as possible from the leaves. This was done to facilitate isolation of the protein. *Starch hydrolysis was considerably retarded in the minus-nitrogen leaves.*

Leaves were sampled immediately after removal from the dark, inoculated, and the check halves individually weighed and adjusted to equal weights. The results (Fig. 2) show that in the minus-nitrogen plants (a trace of residual nitrogen may have been present in the culture medium) and in the medium-nitrogen plants, synthesis of virus protein had proceeded at the expense of the chromoprotein. At the high-nitrogen level, however, only a slight reduction in chromoprotein occurred, in spite of an increased synthesis of virus protein. Biological assays of virus activity corroborated the chemical data. Although the least amount of virus was found at the low-nitrogen level, the symptoms appeared earlier and were much more pronounced than at the medium-nitrogen level. It is particularly significant that the actual area invaded was apparently about the same in both cases. At the high-nitrogen level the symptoms remained almost completely masked, notwithstanding the high virus titre. Protoplasmic streaming

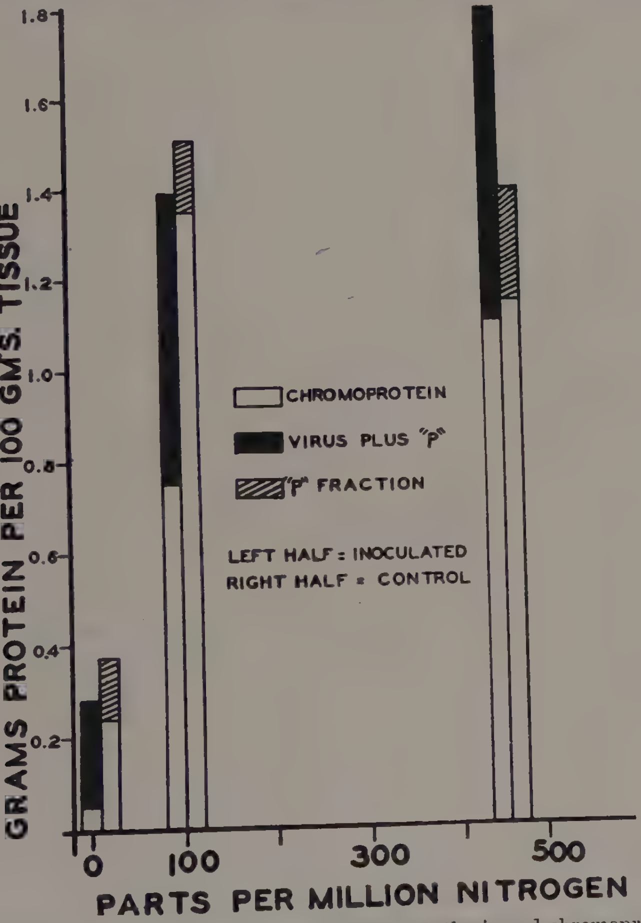


FIG. 2. The influence of nitrate supply on virus synthesis and chromoprotein content in half leaves of Turkish tobacco. Samples were collected 8 days after inoculation. The procedure followed in obtaining the different fractions is given in table 1.

measurements of the control halves 3 days after inoculation showed that the cyanide-sensitive respiratory system ("A-system") had been greatly reduced in activity in the low-nitrogen plants and was very active in the high-nitrogen plants. Other similar experiments indicated the same general relationships in regard to the proteins and cell metabolism.

Experiments with detached leaves of Turkish tobacco indicated that virus protein is synthesized at the expense of chromoprotein. In one experiment, 75 mg. of virus protein was synthesized in 100 g. of fresh leaf tissue in 4 days. The control halves of the same leaves yielded 453 mg. of chromoprotein per 100 g. of tissue, whereas the diseased tissues yielded only 379 g. of chromoprotein. The reduction in chromoprotein was thus equivalent to the amount of virus protein formed.

While systemically infected plants grown at intermediate nitrogen levels indicated a replacement of chromoprotein by virus protein it was, of course, necessary to use separate plants as controls. In one experiment, in a plant infected for approximately 60 days with a "yellow strain" of the virus, only 185 mg. of chromoprotein were present in 100 g. of fresh leaf tissue, although approximately 180 mg. of virus protein were isolated. A healthy plant of similar age, and grown under the same conditions, yielded approximately 300 mg. of chromoprotein. In another experiment leaves from a Turkish tobacco plant, systemically infected with the "green strain" of virus, yielded approximately 748 mg. of virus protein per 100 g. of fresh leaf and about 1100 mg. of chromoprotein. A comparable healthy plant, grown under the same conditions, yielded 1704 mg. of chromoprotein per 100 g. of leaf. These plants were grown at a relatively high nitrogen level, the control leaves being very dark-green. This yield of virus is the largest obtained in any experiment. The large yield of chromoprotein was due to the fact that relatively young leaves with closely packed cells, rich in chloroplasts, were sampled.

DISCUSSION

The results and conclusions presented in this paper indicate that the inter-relations between inorganic nitrogen supply to the cell, respiration, chromoprotein synthesis, and virus protein synthesis can be represented as in figure 3. As Spencer (14, 15) has pointed out, the virus behaves as a foreign protein in that the cell is unable to bring about its hydrolysis. That the virus is not necessarily toxic in itself, however, is indicated by the masking of primary symptoms that occurred in high-nitrogen plants during formation of large amounts of virus protein. *The extent of injury caused by the virus seems to be dependent on the ability of the cell to maintain its cyanide-sensitive respiratory enzyme system and normal chromoprotein balance. The inorganic nitrogen supply to the cell is a major factor in maintaining this balance, although without inorganic nitrogen, virus synthesis still proceeds.* Woods (17) many years ago pointed out that symptoms of tobacco mosaic are in part those of nitrogen starvation.

Our results indicate that virus protein is synthesized in a manner closely paralleling the synthesis of chromoprotein. The data indicate that the virus may be formed from the same building units for chromoprotein (or its hydrolytic products) if not from the chromoprotein itself. Holmes (7) has shown that chlorophyll *per se* is not necessary for virus multiplication. This observation in no way invalidates the hypothesis just expressed. In the first place, plastids, especially leucoplasts, may occur in all plant organs. Secondly, Dufrenoy and Dufrenoy (5) Guilliermond *et al* (6) have shown that mitochondria (which are always present in the cell) develop into plastids and may thus be similar to plastids with respect to certain protein components. In this connection it is interesting to note that Claude (3) isolated the protein fraction from the mitochondria of cancerous animal tissue and obtained an elementary chemical analysis almost identical with certain plant viruses, including tobacco mosaic. Most significant is the fact that the mitochondrial protein is a ribose type nucleoprotein. Banga and Szent-Györgi (2) also have mentioned that chloroplast proteins may be related to nucleoproteins.

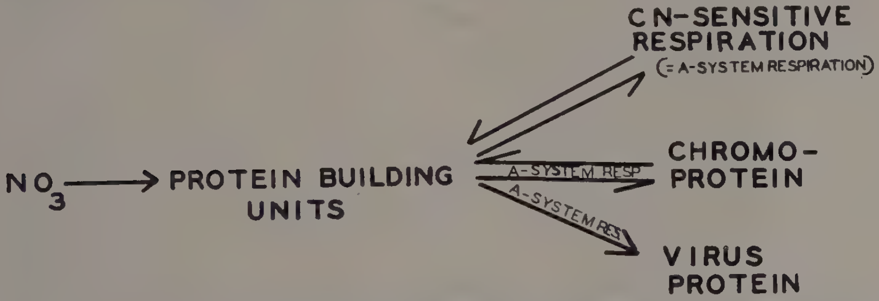


Fig. 3. Diagram representing the interrelation of the activity of a cyanide-sensitive respiratory enzyme system ("A-system") and chromoprotein and virus-protein synthesis, and their dependence upon nitrate supply. Unidirectional arrow towards virus indicates the inability of plant to reutilize virus protein. Function of "A-system" demonstrated by effect of CN on virus synthesis. Indirect evidence exists for a similar functional relationship to chromoprotein synthesis as indicated in the figure.

It is generally admitted that the mitochondria of plant and animal cells probably are very similar in structure and, at least in part, in function (9). If we accept the view of Dufrenoy and Dufrenoy (5), Guilliermond *et al.* (6) and others that plant mitochondria are the progenitors of the plastids, the results of the present study suggest that viruses may be abnormal chondriosomal or chromoprotein derivatives.

SUMMARY

HCN blocks a particular respiratory enzyme system ("A-system") and also blocks virus multiplication.

Nitrogen starvation, if sufficiently prolonged, brings on a reduction in activity of cyanide-sensitive respiration. Since virus multiplication depends on this respiration system, nitrogen starvation can thus prevent or retard virus multiplication.

A method is outlined for the quantitative extraction and separation of leaf chromoprotein and virus protein. Previously undescribed properties of the chromoprotein are presented.

Tobacco-mosaic protein is synthesized in a manner closely paralleling the synthesis of chromoprotein and the data indicate the virus may be formed from the same building units as the chromoprotein itself.

The data suggest that tobacco-mosaic virus may be either an aberrant chondriosomal or chromoprotein derivative or at least have a highly specific relationship to such proteins.

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LITERATURE CITED

1. ANSON, M. L. On Lubimenko extracts of chlorophyll-protein. Science 93: 186–187. 1941.
2. BANGA, I., and A. SZENT-GYÖRGYI. Structure proteins. Science 92: 514–515. 1940.
3. CLAUDE, ALBERT. Particulate components of normal and tumor cells. Science 91: 77–78. 1940.
4. COOK, MELVILLE T. Histology and cytology of sugar cane mosaic. Journ. Dept. Agr., Puerto Rico 9: 5–27. 1925.
5. DUFRENOY, J., and M. L. DUFRENOY. Cytology of plant tissues affected by viroses. Phytopath. 24: 599–619. 1934.
6. GUILLIERMOND, A., G. MANGENOT, and L. PLANTEFOL. Traité Cytologie Végétale. 1195 pp. (Paris). 1933.
7. HOLMES, FRANCIS O. Increase of tobacco-mosaic virus in the absence of chlorophyll and light. Phytopath. 24: 1125–1126. 1934.
8. LORING, H. S., H. T. OSBORN, and R. W. G. WYCKOFF. The ultracentrifugal isolation of high molecular weight proteins from broad bean and pea plants. Proc. Soc. Exp. Biol. and Med. 38: 239–241. 1938.
9. NEWCOMER, EARL H. Mitochondria in plants. Bot. Rev. 6: 85–147. 1940.
10. PETERSON, PAUL D., and H. H. MCKINNEY. The influence of four mosaic diseases on the plastid pigments and chlorophyllase in tobacco leaves. Phytopath. 28: 329–342. 1938.
11. PRICE, W. C., and W. G. WYCKOFF. Ultracentrifugation of juices from plants affected by tobacco necrosis. Phytopath. 29: 83–94. 1939.
12. SMITH, EMIL L., and EDWARD G. PICKELS. The effect of detergents on the chlorophyll-protein compound of spinach as studied in the ultracentrifuge. Journ. Gen. Physiol. 24: 753–764. 1941.
13. SOROKIN, HELEN. Phenomena associated with the destruction of the chloroplasts in tomato mosaic. Phytopath. 17: 363–379. 1927.
14. SPENCER, ERNEST L. Inhibition of increase and activity of tobacco-mosaic virus under nitrogen-deficient conditions. Plant Physiol. 16: 227–239. 1941.
15. ————. Correlation of activity per unit weight of tobacco-mosaic virus with age of lesion. Science 94: 96–97. 1941.
16. WOODS, ALBERT F. The destruction of chlorophyll by oxidizing enzymes. Centralb. f. Bakt. u. Par. Krankheit. II. 5: 745–754. 1899.
17. ————. Observations on the mosaic disease of tobacco. U.S.D.A. Bur. Pl. Indus. Bull. No. 18. 24 pp. 1902.
18. WOODS, MARK W. Reversible inhibition of tobacco-mosaic virus in living cells with 0.0002 molar sodium cyanide. Science 91: 295–296. 1940.
19. ———— and H. G. DUBUY. The influence of tobacco-mosaic virus on cellular oxidation. Phytopath. (Abstract) 31: 25. 1941.
20. ———— and ————. The effect of tobacco-mosaic virus on cellular respiration. Phytopath. (In press).

EXPERIMENTAL CONSIDERATION OF THE MOLD TOXINS OF GLIOCLADIUM AND TRICHODERMA[1]

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INTRODUCTION

The term mold toxin is here employed to designate substances that are products of the normal metabolism of certain molds and that, even in small quantities, are toxic to other microorganisms. Brief notes have been published (16, 17) on mold toxins extracted from culture filtrates of *Gliocladium fimbriatum* G. and A. and of strains of *Trichoderma.* The toxin, isolated in crystalline form, was derived only from the *Gliocladium.* This fungus had been erroneously considered by the writer as a *Trichoderma* in the first paper (17) in which a brief report was given on the isolation and chemistry of the toxin. *Gliocladium,* as well as *Trichoderma,* is capable of attacking and destroying the hyphae of *Rhizoctonia solani* Kühn and other fungi when grown in conjunction on nutrient culture media (14). The toxins appear to be the chemical agents effective in this lethal action.

The principal purpose of this article is to present data on factors determining production, stability, and activity of the crystalline *Gliocladium* toxin (gliotoxin).[3] Some of the results reported here have been obtained incidental to culturing the *Gliocladium* fungus on a large scale for the purpose of securing sufficient gliotoxin for chemical study.[4] Data on the work with *Trichoderma* are included in the section dealing with the preliminary experiments.

REVIEW OF LITERATURE

A mold toxin, penicillin, has been purified by extraction of the culture filtrate of Fleming's Penicillium with ether (3). This toxin resembles that of *Trichoderma* in being easily decomposed by the oxygen of the air (3, 15). Bacteriotoxins that correspond to the mold toxins in their injurious effects towards other microorganisms, have been known for a long time. *Pseudomonas fluorescens* produces a thermostable toxin which is soluble in alcohol (8). The lipoid nature of a bacterio-toxin of *Bacterium pyocyaneus* Gessard has been demonstrated (12). The term "lipoid" indicates merely a solubility relation and tells nothing about other characteristics of the toxins, except that this property may enable them to penetrate rapidly the plasma membrane of living cells. However, the fact that the toxins discussed here are not complex proteins gives hope for isolating other toxins in chemically pure form.

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[3] The term "gliotoxin" has been suggested by J. R. Johnson.

[4] The writer assisted in the initiation of this work at the Baker Laboratory of Chemistry, Cornell University, where W. F. Bruce and J. R. Johnson are investigating the chemistry of gliotoxin.

Apparently the mold toxins have no relation to the classical bacterial toxins that induce the production of specific antitoxins when injected into susceptible animals. According to Eaton (4) the purest preparations of true bacterial toxins give protein tests, while work reviewed by Wells (19) tends to show that they are not proteins. Wells also discusses the ability of some lipoids to modify the antigenic specificity of proteins with which they unite.

Toxic substances of lipoid nature have been shown to play rôles in animal and plant diseases caused by microorganisms. Bodin and Lenormand (1) report that one of the two poisons produced by *Aspergillus fumigatus* is a lipoid. It may be extracted by ether from the mycelium or the culture medium of the pathogen, and is thermostable in acid solution. Clayton (2) has demonstrated that the toxin produced by *Bacterium tabacum* Wolf and Foster is thermostable and is not affected by various protein precipitants, including alcohol. Fusarinic acid (20) has been isolated from the culture filtrate of *Fusarium heterosporum.* It has a growth-inhibiting effect upon rice seedlings.

PRELIMINARY EXPERIMENTS

Before concentrating the work on *Gliocladium,* a search was made among various antagonistic fungi for isolates that would furnish filtrates sufficiently potent and stable for chemical tests. *Gliocladium fimbriatum* gave filtrates characterized by a toxicity that was more stable than that from *Trichoderma* isolates of the pigmented type (15). Nontoxic filtrates were obtained from all other isolates of *Trichoderma* and from *Acrostalagmus cinnabarinus* Corda. Since these organisms destroyed hyphae of *Rhizoctonia* in nutrient agar plates, on contact, as well as at a little distance, it appears very likely that they produce a diffusible toxic agent. Preliminary tests seemed to indicate that the failure to demonstrate toxins was not because of volatility of toxic materials, accumulation on prolonged culture, or dependence upon the presence of other organisms.

As in previous work (15), the fungi were grown in Petri dishes on a liquid glucose-peptone medium at pH 4.5 for two days. The toxicity of the filtrate was determined by suspending agar disks with *Rhizoctonia solani* in dilutions placed on glass slides. The highest dilution at which the contents of the *Rhizoctonia* hyphae were seen to be disorganized on microscopic examination, served as a measure of toxicity (lethal index) corresponding to the minimum lethal dose of bacteriologists. When larger amounts of filtrate became necessary, the fungi were grown in flasks, using the shaking culture method herein described. *Gliocladium* filtrates from shaking cultures were almost as effective as those from Petri-dish cultures. Yields of the *Trichoderma* toxin were not so good as in Petri dishes. Standing flasks were unsatisfactory.

It was thought that anti-oxidants might stabilize the *Trichoderma* toxin whose rapid decomposition is effected mainly by the oxygen of the air (15). Addition to the culture solution of 0.5 per cent (by weight) hydroquinone proved ineffective. However, when ethyl alcohol was added to the culture filtrate of *Trichoderma* to give a concentration of 33 per cent, most of the toxic effect could be preserved for one day and some of it for 3 days.

At the beginning of this investigation, unsuccessful attempts were made by various methods to purify the mold toxins: adsorption on charcoal and kaolin, filtration through a graded series of collodion membranes, and use of protein precipitants. The aforementioned stabilizing effect of ethyl alcohol suggested the use of alcohol for extracting the toxins. Ethyl alcohol was added to the culture filtrate of *Gliocladium* to give a concentration of 33 per cent alcohol. When salted out by saturating with ammonium sulphate, the supernatant alcoholic phase was found by toxicity test to contain more than half of the toxic materials of the original filtrate. The residue left after evaporating the alcohol dissolved sparingly and slowly in water, but redissolving it in a little 95 per cent alcohol proved to be an easy means of bringing it into the water solutions.

TABLE 1.—*Extraction of culture filtrates of Gliocladium and Trichoderma (Volume of solvents = 40 per cent of volume of filtrate)*

Solvent	Lethal index[a] of						Control	
	Gliocladium filtrate			Trichoderma filtrate				
	Original	Solvent phase	Water phase	Original	Solvent phase	Water phase	Solvent phase	Water phase
Chloroform	50	40	2	90	60	6	4	1
Ethyl ether	50	30	15	90	40	22	0	2
Benzene	50	32	3	......	......	......	1	1
Petroleum ether	50	2	40	90	2	60	0	2

[a] Lethal index 50 means that 1:50 was the highest dilution at which most *Rhizoctonia* hyphae died.

Extraction by lipoid solvents other than alcohol demonstrated the lipoid nature of the 2 mold toxins (Table 1). Chloroform proved to be a more effective extracting agent than did ether or benzene, while petroleum ether did not remove the toxins. The toxicity data were obtained in these experiments at dilutions containing 0.2–2.0 per cent alcohol. These high dilutions excluded the possibility of alcohol being the toxic factor. Dilutions were made in tenth molar solutions of KH_2PO_4, which were toxic to *Rhizoctonia* hyphae at an alcohol content of 10 per cent, but not at 5 per cent. The solvents were evaporated from the extracts by applying a vacuum at room temperature and from the watery phase by a stream of carbon dioxide to avoid destruction of the *Trichoderma* toxin. The minimum lethal dose of the crude *Gliocladium* toxin obtained as residue of the chloroform extract was calculated as 3 to 5 p.p.m., while that of the crude *Trichoderma* toxin was 50 to 100 p.p.m. It should be considered that the instability of the latter no doubt caused a considerable loss during the processes of extracting and testing.

FACTORS AFFECTING THE PRODUCTION OF GLIOTOXIN

Methods

A modification of the shaking-culture method of Kluyver and Perquin (7) gave better results in this work than any other procedure tried. Since the method seems to be little-known, it will be discussed in some detail. Data on physiological work with mat-forming fungi frequently show excessive variations between duplicate flasks. The mycelial mat of fungi, grown on a liquid medium by the conventional method, is composed of cells originating and living under heterogeneous conditions, particularly with respect to aeration and nutrition. The culture period extends over a long time, so that some mycelia are growing while others form spores or die. By placing the culture flasks in a shaker, Kluyver and Perquin secured homogeneous cell material in the form of mycelial balls. Aliquot amounts of these balls were transferred after 2 days' growth to media in other shaking flasks for physiological experiments. Uniform and abundant supply of nutrients and rapid diffusion of metabolites hastened growth, so that good yields of kojic acid were obtained from *Aspergillus flavus* within a day, and duplicate flasks checked closely (7).

The primary purpose of the work on the *Gliocladium* was to secure considerable quantities of crystalline material for chemical study. The best yields were obtained at high shaking speeds, 90 to 100 movements per minute, instead of the 80 used in the original method. Through such violent shaking, diffuse mycelium developed in place of mycelial balls. The cultures were grown from spores, since transfer of mycelium from one flask to the other after 1 or 2 days' growth reduced the toxin yield greatly.

The general procedure used at first will be outlined here. Modifications are discussed with the experimental data. Round, 500-cc. and 1000-cc. culture flasks with long necks were filled to $\frac{1}{3}$ their volume with the following medium: 25 g. glucose, 2.5 g. peptone, 2 g. KH_2PO_4, 1 g. $MgSO_4$, and .01 g. $FeCl_3$ in 1000 cc. water. The pH of the medium was adjusted to 3.5 by adding phosphoric acid prior to autoclaving for 30 min. at 15 lb. pressure. Spore suspensions for inoculation were prepared by washing spores from potato dextrose agar slants on which the fungus had grown for 6 to 10 days. The concentration of the suspension was determined by means of a spore-counting cell. Measured amounts of the suspension were pipetted into the flasks just before placing them on the platform of the shaker. The latter stood in a room of fairly uniform temperature (23° C. ± 2). At the conclusion of the shaker runs, which usually lasted 2 to 3 days, the mycelia were separated from the culture filtrate by passing through filter paper. The toxicity of samples was determined by the aforementioned biological test. Then, 2 extractions were made by chloroform, using amounts equal to 10 per cent of the water phase. After distilling the chloroform from the extract over a water bath, the residue was taken up in a little hot ethyl alcohol from which the gliotoxin crystallized on cooling. A second crystalline fraction usually was obtained from the concentrated mother liquors. The combined yields were dried in a desiccator and weighed. The combined mother liquors also were dried to a gum and weighed.

Time Relations in Toxin Production

Most of the gliotoxin is produced within the first 2½ days of growth under the aforementioned conditions. Figure 1 shows that the toxicity of the filtrate does not increase after that time. The yields of gliotoxin decreased in this experiment after 2½ days. This probably was due to the appearance in the chloroform extracts of progressively larger amounts of gum, which hindered purification of the crystalline material. In later work, ammonium salts instead of peptone, were used as the source of nitrogen, because little gum was formed with them. Cultures grown 3 days in ammonium media yielded usually a little more toxin than comparable cultures grown for 2 days. The curve of yield data of gliotoxin obtained from such media probably would be similar to that of the lethal indices of the filtrate in figure 1.

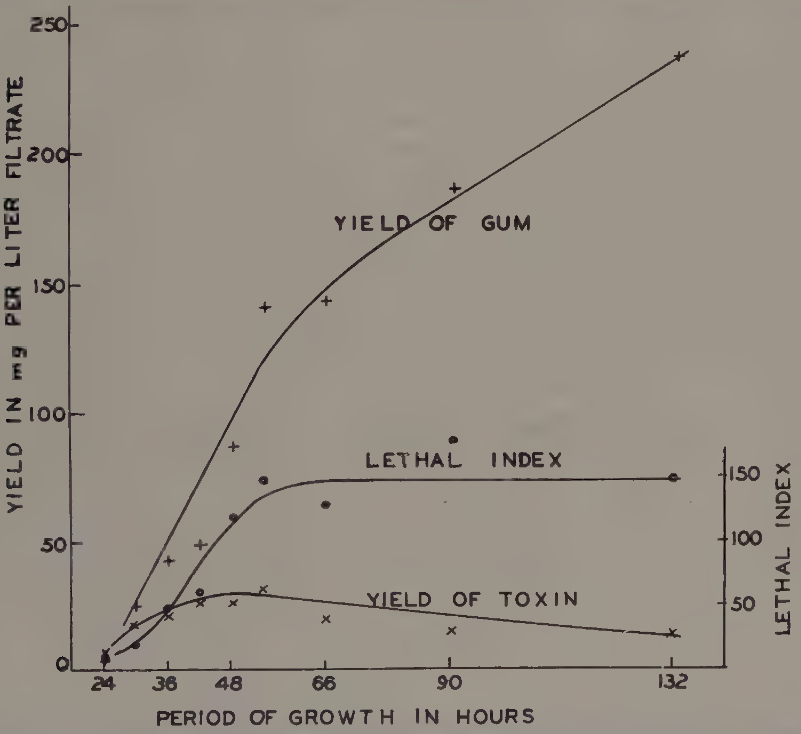

FIG. 1. Production of gliotoxin in a glucose-peptone medium of pH 3.5. Toxicity of filtrates from cultures grown by the shaking method for various periods, and amounts of toxin and gum extracted from them by chloroform.

Each of the yield data of figure 1 was derived from twelve 500-cc. flasks. The yield calculations were made on the basis of the volume of filtrate obtained after 36 hours' growth. The pH of the culture medium at the conclusion of the runs varied between pH 3.2 and 3.6 The inoculum consisted of 100 million spores per flask.

Effect of Amount of Inoculum on Toxin Production

The growth period at which the gliotoxin is produced in shaking cultures is the period of most rapid multiplication of cells corresponding to the phase of logarithmic increase in the growth of bacterial cultures. The logarithmic phase is limited in any given volume of culture medium by a certain cell population. It was thought that lowering the initial population might extend the logarithmic phase and increase toxin yields. Table 2 indicates a definite trend in this direction. Thimann and Dolk (13) have shown that the production of indole-acetic acid by *Rhizopus suinus* is independent of the amount of inoculum over a wide range. This substance is released by the fungus in the period after logarithmic growth.

TABLE 2.—*Effect of amount of inoculum on the yield of gliotoxin (fungus grown for 36 hours in glucose-peptone medium at pH 3.5)*

Experiment number	Number of spores per 500-cc. flask	Yield per 1000 cc. of filtrate	
		Pure toxin	Gum
	Million	*mg.*	*mg.*
1	100	19.2	42.0
	10	27.8	35.2
2	80	14.7	102.5
	20	17.7	52.1
	5	29.6	33.0

Effect of pH and Composition of the Culture Medium on Toxin Production

Production of gliotoxin was considerably smaller at low hydrogen-ion concentrations than at high ones. In a representative experiment, the culture medium of one-half of the flasks was adjusted to pH 3.5, that of the other half to pH 6.0. After two days' growth the pH values were 3.5 and 5.9. The lethal indices of the filtrates were 65 and 7, and the toxin yields were 28.1 and 2.5 mg., respectively, per 1000 cc. of filtrate.

The weights of fungus mycelia obtained in this experiment were determined after thorough drying. The mycelia produced at pH 6.0 weighed nearly twice as much as those of pH 3.5. Results obtained in other experiments confirmed that no correlation existed between yields of mycelium and toxin.

Apparently, the yield of toxin was not affected in media containing double or half the amounts of ingredients of the normal. Ammonium tartrate, replacing peptone as nitrogen source, gave higher yields (40–70 mg.

per l.), possibly because of the scarcity of gum material in the chloroform extracts. Further experiments regarding the effect of nutrients on the production of gliotoxin were carried out in connection with large-scale cultivation of the fungus. The organism was grown for 3-day periods in 3- or 6-liter flasks, filled to $\frac{1}{2}$ their volume with culture medium, and placed on a shaker carrying about 50 liters at a time. Very good toxin yields (40–100 mg. per l.), were obtained at reactions of media as acid as pH 2.5–3.0. This high acidity allowed the use of media prepared without complete sterilization. Thus was avoided the need of such expensive equipment as sterilizer-incubators, which have been used for work of this type in various forms (6, 11). Contaminations were rarely observed when hot tap water of 60–70° C. was used in making the medium, and when vessels and utensils were kept rigorously clean. In a few cases cultures had to be discarded because a red yeast contaminant developed. In spite of this unorthodox method of cultivating the fungus, the following results are considered fairly well established. Glucose could be replaced by sucrose, but not by glycerine or levulose. Ammonium salts gave much better results than nitrates. This difference was apparently not attributable to pH relations, for the pH of the nitrate media did not rise above 3.1.

The standardization of aeration and shaking practices should be carried farther than was done in this work if it is desired to secure uniform results. Duplication of results on production of gliotoxin was difficult. Occasionally, considerable differences were obtained between duplicate sets of cultures grown at the same time. Such variations were common with sets of cultures grown at different times. The shaking-culture method has, nevertheless, proved useful for toxin production because it speeds up the growth cycle. Most of the mycelia pass through the logarithmic phase of growth within such a short period that the toxin may be secured without interference from other chloroform-soluble substances that accumulate on prolonged culture.

The Mechanism of Toxin Production

Since it can be obtained on synthetic media lacking organic radicals, except those of sugar, gliotoxin must be either synthesized from sugar and salts, or broken down from other compounds built up by the fungus from sugar and salts. The toxin apparently is not a decomposition product of proteins, as might be inferred from the presence of nitrogen and sulphur in the molecule. Methods used with endo-toxins have failed to secure it, such as autolyzing the mycelium, and extracting the dried and ground hyphae with water or lipoid solvents. Moreover, the amount of toxin produced does not increase after the period of logarithmic growth, as would be expected of a break-down product of proteins. Since this is true also under conditions giving relatively small yields, it cannot be attributed to saturation of the solution with respect to the material.

The yield curve of the gliotoxin in shaking cultures indicates that the peak of production is around the middle of the second day of growth. The pathogenic effect of *Gliocladium* is found to be also at its height during the second day, when the fungus is planted in nutrient agar cultures by the seeded-plate method, and grown in conjunction with *Rhizoctonia solani* at 25° C. The period of maximum toxin production and the attack on other fungi are slightly earlier in this case than those observed with *Trichoderma* (15).

It appears that abundant supply of oxygen and high concentration of hydrogenions are conditions essential to good yields of the toxin. The composition of the medium appears to be of secondary importance, though ammonium salts are more favorable than other nitrogen sources. The significance of these findings with respect to the mechanism of production of the toxin may become clear through the investigation of its chemistry.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON STABILITY AND ACTIVITY OF GLIOTOXIN

Method of Toxicity Test

The method of testing the toxicity of culture filtrates was mentioned at the beginning of this article. The following modification was used in experiments with gliotoxin. *Rhizoctonia* cultures on non-nutrient agar were cut into square pieces of 5 mm. diameter. Four such pieces were mounted on a glass slide, each one in 2 drops of the test solution. Four glass slides were used for each dilution. The slides were incubated in moist chambers at 27° C. for 20–24 hours. The L.D. 50 (dilution at which 50 per cent of the hyphae survived) was computed by plotting the toxicity percentages obtained at the various dilutions and drawing the toxicity curve.

This is an adaptation of the method McCallan and Wilcoxon (10) used in their extensive work on spore germination and the effect of fungicides. Objections may be raised against the *Rhizoctonia* test on the grounds that toxicity data are based upon estimates of the percentage of hyphae affected. Corresponding toxicity tests have been carried out, therefore, using the favorite test fungus of McCallan and Wilcoxon, *Sclerotinia americana* (Worm.) Nort. and Ezek. Spore germination counts were made strictly according to their method.

Results of tests comparing the effect of gliotoxin with that of metallic fungicides were very similar for *Sclerotinia* and *Rhizoctonia* (Tables 3 and 4). The toxicity of mercuric chloride to *Rhizoctonia* was about 1.5–3 times that of gliotoxin, while the difference tended to be slightly smaller with *Sclerotinia*. Gliotoxin was very much more toxic than copper sulphate, particularly with *Rhizoctonia*. Based on molecular concentrations, the relative values should be still more in favor of the toxin, since it has the higher molecular weight.

The germination of *Gliocladium* spores in solutions of the same materials presents an interesting contrast (Table 4). The two metallic fungicides were about as effective on *Gliocladium* as on *Sclerotinia*. Inhibiting effects of the toxin on germination of the *Gliocladium* spores were so slight, how-

TABLE 3.—*Relative toxicity of metallic fungicides and gliotoxin toward Rhizoctonia solani*

Chemical	Experiment number				
	1	2	3	4	5[b]
Gliotoxin	300[a]	425	250	250	630
Mercuric chloride	610	600	700	750	800
Copper sulphate	170	....	23	40	105

[a] Toxicity expressed as LD 50 × 1/1000, that is, at a concentration of 1: 300,000, 50 per cent of the *Rhizoctonia* hyphae survived.

[b] Test in glass vials instead of on glass slides.

TABLE 4.—*Relative toxicity of metallic fungicides and gliotoxin toward spores of Sclerotinia americana and Gliocladium fimbriatum*

Chemical	*Sclerotinia americana* experiment No.					*Gliocladium f.* experiment No.	
	1	2	3	4	5	1	2
Gliotoxin	458[a]	292	603	353	413	Below 15	Below 15
Mercuric chloride	478	681	1068	532	912	700	1222
Copper sulphate	235	....	352	174	262	90	142

[a] Toxicity expressed as LD 50 × 1/1000, that is, at a concentration of 1: 458,000, 50 per cent of the spores germinated.

ever, that they could not be expressed accurately in table 4. The highest concentration of the toxin used was 66.7 p.p.m. which represents nearly a saturated solution in distilled water, and thus limits the observable toxicity for this method. Toxicity tests with potent culture filtrates of *Gliocladium* showed a similar lack of activity upon both the fungus and its spores.

Tables 3 and 4 reveal considerable variations between experiments carried out at different times. McCallan and Wilcoxon (9) have called attention to this difficulty, one that has not been entirely overcome by extreme care in standardizing conditions. This fact should not be overlooked in making comparisons of the data.

Representative experiments have been selected for the following sections. These experiments were carried out after the effective ranges of the treatments had been determined in preliminary tests.

Stability of the Gliotoxin

Aliquot amounts of the solution containing 66.7 p.p.m. of the pure toxin were adjusted to various pH values by adding molecular potassium phosphate buffer solutions at the rate of 1 part to 20 of the toxin solution. The samples were stored or heated in sterile test tubes closed with cotton plugs. Buffer solutions of pH 4.5 were used when testing the toxicity.

The acidified solutions preserved their toxicity for long periods, confirming previous results with nonpurified toxin. In alkaline solutions, however, the rate of decomposition increased decidedly with increasing pH (Table 5). More of the toxicity was destroyed after 1 hour at pH 9.0 than

TABLE 5.—*Effect of pH on the stability of solutions of gliotoxin (Toxin solutions kept at the pH values during specified periods at 27° C., before testing them at pH 4.5)*

Experiment number	Period of storing	Toxicity after storing in buffer solution of pH					Control (original solution)
		4.5	6.0	7.1	8.2	9.0	
1	1 hour	....	....	420[a]	360	120	360
	6 hours	....	....	....	122	28	....
	24 "	390[a]	....	....	22	Below 15	....
2	1 hour	....	....	300	330	22	300
	6 hours	....	....	....	180	Below 15	....
	24 "	300	....	....	28	" 15	....
3	3 days	405	....	375	....	....	670
	14 "	620	....	Below 15	....	....	....
4[b]	7 days	600	320	240	....	....	640

[a] See table 3, footnote a.
[b] Solutions sterilized by ultrafiltration before storing.

after 6 hours at pH 8.2, or 24 hours at pH 7.1. The loss of activity at pH 7.1 in experiment 3 was probably due to the effect of bacterial contaminants that developed in the solution. Decomposition proceeded very slowly in the solutions of pH 7.1, which had been sterilized by passing through a Berkefeld filter (Experiment 4). Samples of pH 7.1 stored at 37° C., instead of 27° C., lost from $\frac{2}{3}$ to $\frac{3}{4}$ of their toxic effect within 24 hours, according to tests paralleling those presented in table 5. This indicates that the rate of the reaction involved in decomposition increases rapidly with rising temperature. The toxicity data at pH 4.5 (Experiment 3) bring out the need of adequate controls when tests have to be made at different times. Apparently, the material used after 3 days was less sensitive than that at the beginning and at the conclusion of this experiment.

The thermostability of the toxin was found to depend on the acidity of the solution (Table 6). Boiled in a water bath, the solutions decomposed at pH values below 5.0 much more slowly than at less acid pH levels. At pH 2.4, the toxin was almost stable, even when autoclaved for 30 minutes at 16 lb. pressure.

TABLE 6.—*Effect of heating at various pH values on solutions of Gliotoxin. (Tested at pH 4.5)*

Heating		Heated in buffer solutions of pH					Original solution
Temp. °C.	Time minutes	2.4	4.5	5.0	5.6	6.1	
100	15	360[a]	360	310	45	30	360
	30	360	360	210	22	22	....
	60	360	195	90	22	15	....
123	30	330	100	22	15	Below 15	....

[a] See table 3, footnote a.

Activity of the Gliotoxin

As is usual with inhibiting substances, the action of toxin solutions was increased and accelerated with rising temperatures (Table 7). The tests were carried out in screw-top vials containing 3 cc. of solution into which the agar disks with *Rhizoctonia* were placed. This procedure proved to be more practical under the conditions of the experiment, and gave results similar to the glass slide method.

TABLE 7.—*Effect of temperature on the activity of gliotoxin. (Tests in screw-top glass vials at pH 4.5)*

Period of test, hr.	Temperature during test in °C.						
	3	12	18	21	27	30	32.5
4	....	....	....	....	15[a]	55	215
8	....	....	35	85	180	215	925
20	20[a]	45	185	305	380	500	1200
28	40	80	305	....	....	....	....
48	120	160	....	....	....	....	....

[a] See table 3, footnote a.

Note: blank spaces at upper left indicate toxicity values below 15, those at lower right indicate that no further increases in toxicity were obtained.

Tests of toxin solutions at various pH levels showed that maximum activity occurred at pH 8.2 (Table 8). At pH 9.5 the toxin was destroyed. The high toxicity at pH 8.2 may be attributed to an intermediate product in decomposition. It was noted that toxin solutions became deep yellow shortly after adding alkali, and changed to a pale yellow later.

TABLE 8.—*Effect of pH on the activity of the gliotoxin*

Experiment number	Test in buffer solutions of pH			
	4.5	7.1	8.2	9.5
1	340[a]	320	640	....
2	540	400	1200	....
3[b]	360	390	900	Below 15
4[b]	300	300	1575	Below 15

[a] See table 3, footnote a.

[b] Tests in glass vials.

It has been shown with respect to the stability of the toxin that temperature and pH effects have to be considered jointly. Further investigation will be necessary to complete our knowledge in regard to the effect of these factors on the activity of the toxin.

The isolation of the gliotoxin makes it possible to study the effect of environmental factors on the chemical agent responsible for the antagonistic effect of one fungus toward the other. Such studies seem desirable if one wishes to understand and to make practical application of the mechanism of these interactions. In this investigation, production, as well as stability, of the toxin were found dependent upon the acidity of the substratum, while activity increased with rising temperature. If it is assumed that such behavior may be true in general for antagonistic organisms in the soil, one might thus explain why the antibiotic activity of these organisms toward plant pathogenic fungi in various experiments has been found to be favored by high temperature and high acidity of the soil (5, 18). Since the toxin is formed by the *Gliocladium* during active growth only, its antagonistic activity in soil evidently is limited by the supply of organic materials the fungus has to share with numberless competitors of the soil population. When viewed thus against the background of nature's associations, the present study is a small beginning in the direction of elucidating association effects of microorganisms.

SUMMARY

In experiments leading to the isolation of the crystalline toxin of *Gliocladium* (gliotoxin), fungi, antagonistic to *Rhizoctonia solani* in nutrient culture media, were investigated with respect to the toxicity of their culture filtrates. Potent filtrates were secured from *Gliocladium fimbriatum* and from certain *Trichoderma* isolates, but not from other *Trichoderma* isolates. The toxic substances could be extracted from filtrates of the two fungi by lipoid solvents. Chloroform was the most effective extracting agent.

The gliotoxin is produced during the logarithmic phase of growth. Also, it is to be noted that the antagonistic action on other fungi, when grown in conjunction with *Gliocladium,* takes place at this period. The toxin is thought, therefore, to be the agent responsible for the antagonistic effect. The shaking-culture method, which has given high yields of toxin, speeds up the life cycle of the fungus. This facilitates the purification of the toxin, since other chloroform-soluble substances accumulate on prolonged culture, particularly in media containing peptone.

Abundant supply of oxygen and high acidity (pH 5.0 and lower) appeared to be essential for good yields of gliotoxin. Ammonium salts as nitrogen source gave better results than peptone or nitrates. Glucose and sucrose were good carbon sources, while levulose and glycerine depressed yields. No correlation was found between amounts of mycelium and toxin produced. Nonsterilized media of pH 2.5–3.0 were employed for large-scale production of the toxin. The high acidity minimized the influence of contaminants during the brief growth period of 3 days.

Toward the germinating spores of *Sclerotina americana* and hyphae of *Rhizoctonia solani,* the fungicidal effect of gliotoxin is greater than that of $CuSO_4$ and less than that of $HgCl_2$. The germination of spores and vegetative activity of the mycelium of *Gliocladium* are little affected by nearly saturated solutions of the toxin.

In neutral and acid solutions, the toxin is stable when kept for several weeks at room temperature. In alkaline solutions, the toxin is very unstable. The rate of decomposition increases with increasing alkalinity and tempera-

ture. Toxin solutions of pH 2.4 are not affected by exposure to 122° C. for 30 minutes. The thermostability decreases with decreasing acidity, particularly beyond pH 5.0.

Magnitude and rate of the toxic effect increase with increasing temperature. When tested at various pH levels, maximum activity was noted at pH 8.2. The material was so unstable in a solution of pH 9.5 that toxicity was lost immediately.

LITERATURE CITED

1. BODIN, E., and C. LENORMAND. Recherches sur les poisons produits par l'*Aspergillus fumigatus.* Ann. Pasteur **26**: 371–80. 1912.
2. CLAYTON, E. E. Relation of the toxin produced by *Bacterium tabacum* to the pathogenicity and host range of this organism. Phytopath. (Abstract) **23**: 6. 1933.
3. CLUTTERBUCK, P. W., R. LOWELL, and H. RAISTRICK. Studies in the biochemistry of micro-organisms. Biochem. Jour. **26**: 1901–1918. 1932.
4. EATON, M. D. Recent chemical investigations of bacterial toxins. Bact. Rev. **2**: 3–45. 1938.
5. GARRETT, S. D. Factors affecting the pathogenicity of cereal foot-rot fungi. Biol. Rev. **9**: 351–361. 1934.
6. HERRICK, H. T., R. HELLBACH, and O. E. MAY. Apparatus for the application of submerged mold fermentations under pressure. Ind. Eng. Chem. **27**: 681–683. 1935.
7. KLUYVER, A. J., and L. H. C. PERQUIN. Zur Methodik der Schimmelstoffwechseluntersuchungen. Biochem. Zeits. **266**: 68–81. 1933.
8. LEWIS, I. M. Bacterial antagonism with special reference to the effect of *Pseudomonas fluorescens* on spore forming bacteria of soils. Jour. Bact. **17**: 89–103. 1929.
9. MCCALLAN, S. E. A., and F. WILCOXON. The precision of spore-germination tests. Contr. Boyce-Thompson Inst. **4**: 233–243. 1932.
10. ———, and ———. Fungicidal action and the periodic system of the elements. Contr. Boyce-Thompson Inst. **6**: 479–500. 1934.
11. PETERSON, W. H., *et al.* Large-scale laboratory cultivation of molds. Ind. Eng. Chem. **25**: 213. 1933.
12. RAUBITSCHEK, H., and V. K. RUSS. Zur Kenntnis der bakteriziden Eigenschaften der Pyocanase. Zentr. Bl. Bakt. I. Abt. **48**: 114–122. 1909.
13. THIMANN, K. V., and H. E. DOLK. Conditions governing the production of the plant growth hormone by *Rhizopus* cultures. Biol. Zentr. Bl. **53**: 49–66. 1933.
14. WEINDLING, R. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopath. **22**: 837–845. 1932.
15. ———. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopath. **24**: 1153–1179. 1934.
16. ———. Isolation of toxic substances from the culture filtrates of *Trichoderma* and *Gliocladium.* Phytopath. **27**: 1175–1177. 1937.
17. ———, and O. H. EMERSON. The isolation of a toxic substance from the culture filtrate of *Trichoderma.* Phytopath. **26**: 1068–1070. 1936.
18. ———, and H. S. FAWCETT. Experiments in the control of Rhizoctonia damping-off of citrus seedlings. Hilgardia **10**: 1–16. 1936.
19. WELLS, H. G. The chemical aspects of immunity. The Chemical Catalogue Company (New York). 1929.
20. YABUTA, T., K. KAMBE, and T. HAYASHI. Biochemistry of the "bakanae" fungus. Jour. Ag. Chem. Soc. Japan **10**: 1059–1068. 1934.

THE LINE PATTERN VIROSIS OF THE GENUS PRUNUS[1]

DONALD CATION

(Accepted for publication February 15, 1941)

INTRODUCTION

Within recent years a number of leaf-mottling viroses from various sources have been described for the genus *Prunus.* Confusion as to the identity of these viroses has resulted largely from the lack of standardization of the varieties from which symptom expressions are described and, to a lesser extent, from the varied reactions of the diseased trees growing under different environmental conditions. There are also indications that certain investigators have been working with mixed viroses.

There is some doubt as to the relationships of several diseases of plums and peaches. These are variously described by Valleau (6) as a virus disease of plum and peach, by Thomas and Hildebrand (4) as a virus disease of prune, and by Thomas and Rawlins (5) as the Vacaville plum mosaic. The disease reported by Thomas and Hildebrand was later named prunus virus No. 6 by Smith (3). This paper reports experiences with somewhat similar viroses of plum, peach, and Mahaleb cherry.

VIRUS DISEASES OF ABUNDANCE PLUMS IN NURSERIES

In 1933 the writer was asked to test and index Abundance plum trees from 4 widely separated commercial nurseries. For convenience these nurseries will be designated as source 1 (Kentucky), source 2 (Ohio), source 3 (Tennessee), and source 4 (Alabama). The nursery that furnished the source 1 trees was also the one studied by Valleau (6). Three trees from each source were planted 6 ft. apart in the field and a duplicate series was planted in the greenhouse. Bud grafts from trees of the 4 sources were made on bearing Elberta trees (1 each) and into seedling peach trees (2 each). The buds set in the bearing peach trees grew into plum branches and all other grafts grew or made tissue union. In the next 4 years it was apparent that scion wood from sources 3 and 4 transmitted no unusual symptoms to peach trees. The original trees from sources 3 and 4 were uniformly more vigorous and larger than those from the other two sources and were considered healthy by these standards.

The Abundance trees from source 1 bore lighter green, narrow leaves, somewhat curled under at first but gradually assuming normal habit. They remained distinctly chlorotic throughout the summer, in similarity to the plum disease described by Valleau (6) and illustrated in his figure 18. Abundance trees from source 2 appeared normal in color, size, and shape of leaf but were somewhat lacking in general amount of top growth when com-

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pared with trees from sources 3 and 4. Trees from 3 different sources are shown in figure 1.

Buds from sources 1 and 2, grafted into adjacent bearing Elberta peach trees in the college orchard at East Lansing, sprouted and grew during the first season into equal-size shoots about 3 feet long. The shoots were exceptionally vigorous and the leaves appeared normal during the first season of growth, a response similar to the reactions of sprouts from yellows or red suture bud insertions, which show normal growth the first season.

In 1934, the second year, the leaves on the plum sprout from source 1 grafted into Elberta went off color and displayed symptoms identical to those of the original parent tree. This sprout bore a crop of 12 to 15 plums that appeared normal for the variety in size, color, flavor and time of ripening. In the succeeding years very few fruits were borne on this sprout, although the few borne were normal. The graft inoculations from source 1 did not transmit leaf patterns or any observed symptoms to the Elberta leaves or fruit, or to the inoculated seedling peach trees during 3 additional years of observation, 1935, 1936, and 1937. These grafts are pictured in figure 2.

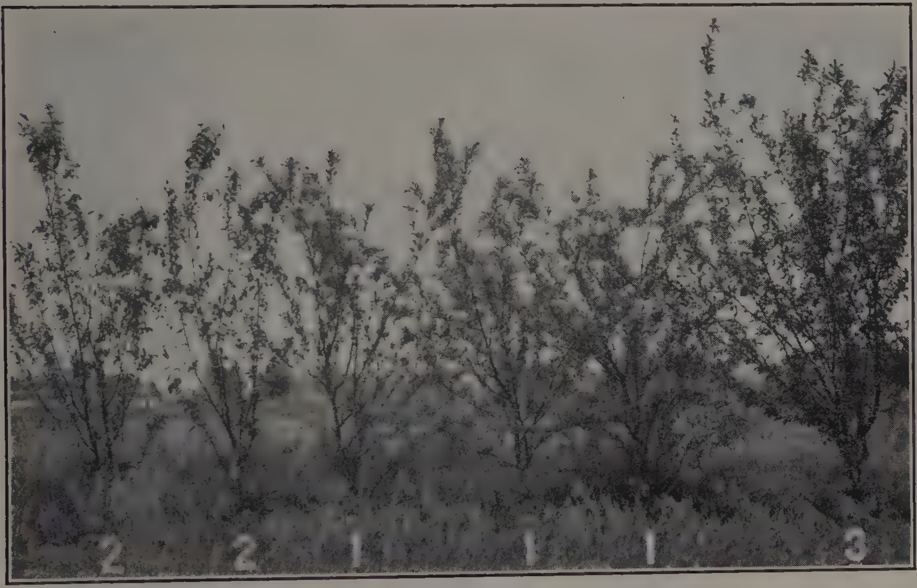

FIG. 1. Abundance plum trees from sources 2, 1 and 3, respectively, in the nursery row.

Comparatively, during the same period, the plum sprout from source 2, grafted into a bearing Elberta tree, bore during the period of observation, some 20 to 30 normal plums annually, and the size, shape, and color of the plum foliage seemed normal for the variety. In further contrast to grafts from No. 1, however, the inoculations from source 2 transmitted line patterns to Elberta and seedling peach trees the year after budding and succeeding

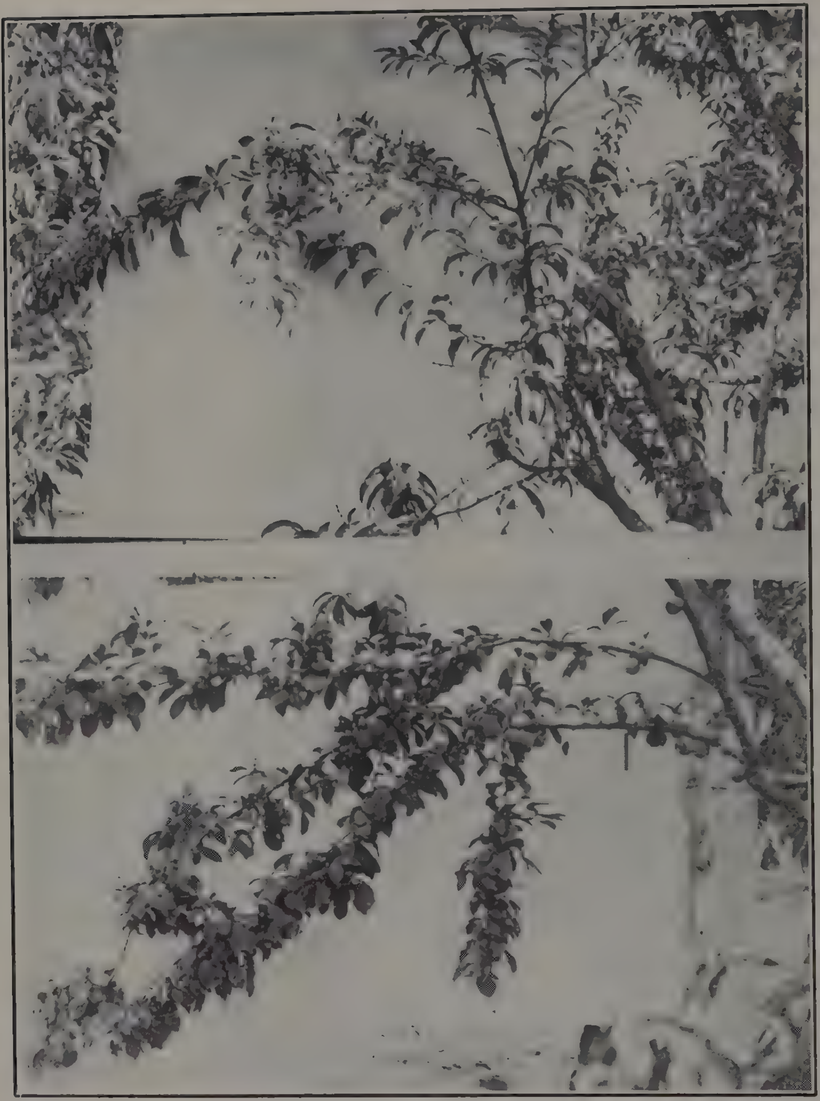

FIG. 2. Plum grafts on peach three years after budding: Top, source 1. This plum branch shows subnormal growth but transmits no symptoms to peach. Bottom: source 2. This plum branch appears normal but transmits line-pattern symptoms to peach.

years. Usually, about a dozen or more plainly marked leaves could be found on each inoculated tree. Some of these trees have been under observation for 6 years, during which time frequent buddings from this source have consistently transmitted line patterns. The differences in the Abundance trees from the 4 different nurseries are presented in table 1.

THE LINE-PATTERN DISEASE IN MICHIGAN ORCHARDS

Attempts to index plum trees in several Michigan orchards for virus diseases showed the line-pattern disease present in 3 widely separated orchards

TABLE 1.—*Differences in Abundance plum trees from four different nurseries*

Source of trees	Characteristics of trees	Effect of inoculation on peach trees
Nursery 1	Smaller, lighter green foliage. Weaker growth, normal fruits but shy-bearing	None observed[a]
Nursery 2	Normal foliage, possibly weaker growth, normal fruits, moderate-bearing	Line-pattern symptoms
Nursery 3	Normal	None observed
Nursery 4	Normal	None observed

[a] Observing similar trees from this same nursery Valleau (6) found that at least part of them transmitted line patterns to peach. Although Valleau recognized the possibility of a mixture of several viruses in this plum stock, his experiences with certain tobacco viroses caused him to lean toward the single virus theory.

on certain Red June and Abundance plum trees. Three other orchards have not produced symptoms. Of the plum trees that transmitted line patterns, mosaic mottlings were observed on only a few leaves of 1 Red June water sprout.

The line-pattern disease has never been observed occurring naturally in Michigan peach orchards, although it appeared naturally on 2 adjacent seedling peach trees in the writer's nursery row. The seedlings were growing near diseased plum trees from source 2, and the escape was presumed to result from an unknown insect vector.

THE LINE-PATTERN DISEASE ON MAHALEB CHERRY

In 1938 the writer found the typical line patterns on Mahaleb cherry sprouts growing from the stock roots of a Montmorency cherry tree near Hart, Michigan. This tree was adjacent to an abandoned plum orchard. No line patterns could be detected in the leaves of the Montmorency cion top. Unfortunately the cherry orchard was cut down before buds were obtained for inoculation. In the same year Abundance plum buds from source 2 were grafted into two Mahaleb seedlings. One of these inoculated Mahaleb seedlings developed definite line patterns in about 12 leaves, which were observed and photographed in August, 1939. Both of the Mahaleb seedlings showed definite line patterns in 1940, as shown in figure 3. Careful examination

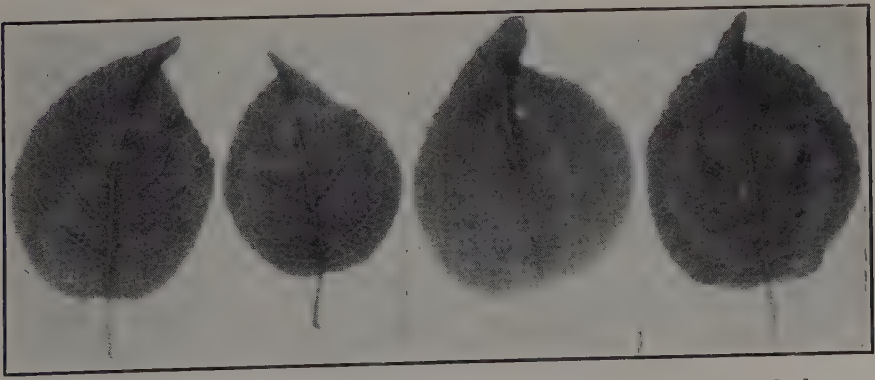

FIG. 3. Line patterns on Mahaleb cherry leaves transmitted from source 2 plum.

showed no line patterns present in 96 other Mahaleb seedlings that served as controls.

OTHER INOCULATIONS OF THE LINE-PATTERN DISEASE

The line-pattern virus was retransferred through several different seedling peach trees by grafting in the past 6 years, with no apparent loss of virulence. The writer has records of 22 positive inoculations by grafting and no negative results when tissue union between diseased and healthy tissue was established. In addition to seedling trees the disease was transmitted to Elberta, Hale, and Carmen varieties, the only standard varieties tried.

SYMPTOMS OF THE LINE-PATTERN DISEASE

The line-pattern virosis of peach is recognized by a diffused type of mottling, rather faint, and of a paler color than the green of the normal leaf. The mottling covers leaf areas of varied size, or is arranged in indefinite crooked lines (Fig. 4). Leaf symptoms are readily detected early in the season, about mid-June, or earlier, but tend to become masked or disappear later in the summer. Some leaves, however, showing line patterns can frequently be found on infected trees throughout the growing season.

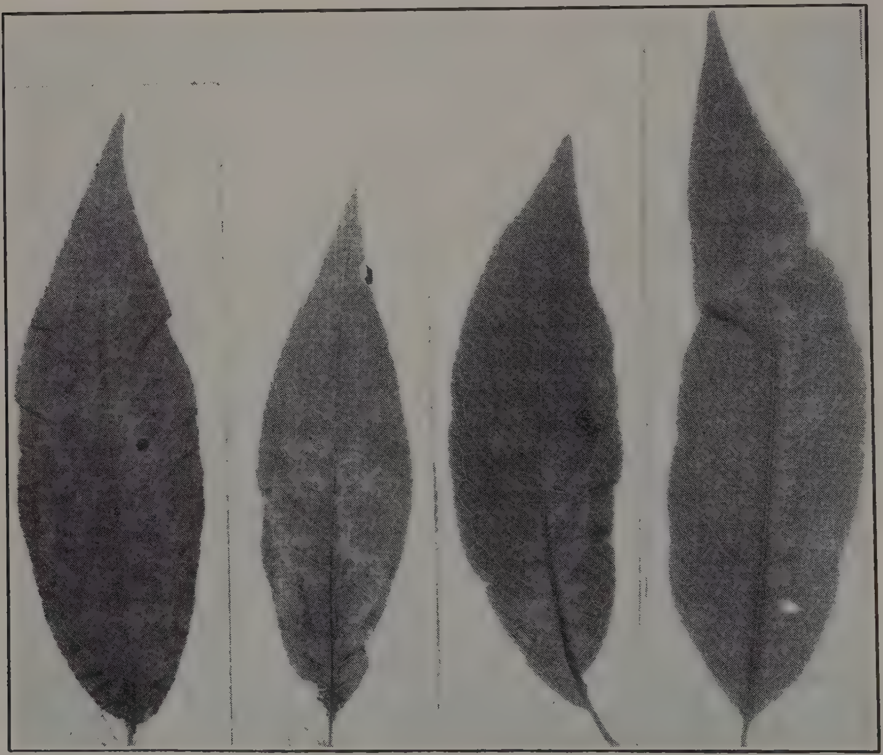

FIG. 4. Line patterns and diffuse mottling on peach leaves transmitted from source 2 plum.

In several instances trees with line-pattern disease could be detected in the nursery rows a dozen or more feet away by a dull cast to the foliage in contrast to a glossy sheen from healthy trees. Neither yellowing of the leaves nor defoliation has been associated with the disease. There is some evidence to indicate that peach trees growing in sod or lacking in vigor are more likely to mask the symptoms. In the writer's experience peach leaves showing the line-pattern disease have never produced necrotic spots, although a spot necrosis of peach, not associated with line patterns, is under observation.

RELATION OF THE LINE-PATTERN DISEASE TO OTHER DISEASES OF THE PEACH AND PLUM

It is apparent from Valleau's (6) illustrations and descriptions that he was working with a line-pattern disease similar to, if not identical with, the one found in Michigan and Ohio. Comparing his inoculation experiences with those in Michigan, it seems possible that he was working with 3 distinct diseases, a line-pattern mosaic, a spot necrosis and a plum virosis somewhat similar to Prunus Virus No. 6. The Vacaville plum mosaic mentioned by Thomas and Rawlins (5) may also be similar to or identical with the peach line-pattern disease. The descriptions and illustrations presented by Atanasoff (1) do not appear identical to the line-pattern disease of peach.

The Abundance trees, here mentioned as from source 1, were from the same source as some of Valleau's (6) trees. These trees had a disease somewhat similar to prunus virus No. 6 in that the leaves were reduced in size and narrowed in proportion to length. They bore but few fruits, and the disease did not transmit to peach. The exact relationship of these diseases could be determined only by grafting them into identical varieties under similar conditions.

NOMENCLATURE OF THE LINE-PATTERN DISEASE

Mild mosaic is not entirely suitable as a name for this disease, for it could then be confused with mild strains of peach mosaic. Vacaville mosaic does not describe it, and it is generally agreed that diseases should not be named after towns or localities.

The name "line-pattern virosis," although not completely descriptive, would serve to identify the disease and separate it from other known maladies of plum and peach. Following the binomial system of Holmes (2) and Valleau's (7) definition of the genus *Marmor*, the following designation is proposed:

Peach Line-pattern Virosis

Marmor lineopictum, New Species. Species name meaning painted in lines.

Susceptible species, *Prunus: P. mahaleb, P. salicina; Amygdalus; A. persica.*

Geographical distribution, Kentucky, Michigan, California, Ohio, and perhaps general over the United States.

Induced disease: In peach and Mahaleb cherry, light colored, line patterns or mottling of indefinite areas, becoming somewhat masked with age of leaf. Frequently, entirely masked in *Prunus salicina.*

Transmission: by grafting.

Serological relationships, immunological relationships, thermal inactivation and filterability, not demonstrated.

SUMMARY AND CONCLUSIONS

Abundance or Red June plums whose leaves appear normal for the variety and that may show little or no mottling or other symptom expressions may transmit a disease to peach trees expressed as a faint mottling of either a line-pattern or diffused type.

The same disease is expressed as line patterns or diffused mottled areas on Mahaleb cherry leaves.

It was shown that the line-pattern disease is carried on certain Abundance and Red June plums in several Michigan orchards.

The line pattern disease was present in certain Abundance plum nursery stock and, from this and similar sources of distribution, it is perhaps widespread on plum trees.

The line-pattern disease on peach appears similar to certain symptoms first described by Valleau and later described by Thomas and Rawlins as the Vacaville disease.

A disease of Abundance plums, with symptoms similar to an unnamed plum disease described by Valleau and perhaps similar to a disease on plums described by Thomas and Hildebrand, did not transmit noticeable symptoms to peach in 3 inoculations.

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LITERATURE CITED

1. ATANASOFF, D. Yearbook of the University of Sofia, Faculty of Agriculture, Sofia, Bulgaria, 13: 9–42. 1935.
2. HOLMES, F. O. Handbook of phytopathogenic viruses. Burgess Pub. Co. (Minneapolis). 1939.
3. SMITH, KENNETH M. A textbook of plant virus diseases. P. Blakiston's Son & Co., Inc. (Philadelphia). 1937.
4. THOMAS, H. EARL, and E. M. HILDEBRAND. A virus disease of prune. Phytopath. **26**: 1145–1148. 1936.
5. ————, and T. E. RAWLINS. Some mosaic diseases of Prunus species. Hilg. 12: 623–644. 1939.
6. VALLEAU, W. D. A virus disease of plum and peach. Kentucky Agr. Exp. Stat. Res. Bull. 327: 89–103. 1932.
7. ————. Classification and nomenclature of tobacco viruses. Phytopath. **30**: 820–830. 1940.

DAMPING-OFF OF LONGLEAF PINE

William C. Davis

(Accepted for publication February 17, 1941)

INTRODUCTION

The longleaf pine (*Pinus palustris* Miller) in its seedling and early sapling years, assumes an unique rosette habit. The symptoms, etiology, and control of damping-off of this species, therefore, present a problem differing somewhat from that for other pines, and calls for special attention. Where damping-off of longleaf pine occurs on sandy soil, some nurserymen speak of it as "sand-splash." When occurring on either sandy or non-sandy soil, Davis *et al.*[1] referred to it as "sand-silt-drift," but admitted the inaptitude of this name. Actually, these designations have little to merit their use, as they refer only to the drifting of surface soil around the base of the seedlings—a single damping-off factor.

The observations and control tests herein reported were made possible through the cooperation of nurserymen of Soil Conservation Service Region 2 and State forest nurserymen of North Carolina, South Carolina, and Georgia.

Symptoms

In the early stage of the attack the bases of the cotyledons, the lower needles, and the hypocotyl of the seedling appear water-soaked and may be of a purplish hue; the uppermost needles and the outermost portion of the lower needles and cotyledons, together with the lower part of the root system, remain apparently healthy. Roots frequently decay rapidly from the soil line to a depth of $\frac{3}{4}$ inch, while the remaining root system decays so slowly as to suggest that its deterioration is associated with organisms other than the initial invader. In the more advanced stage of the disease the needles turn yellow, droop, and eventually become brown. The rosette habit of young longleaf pine seedlings is such that the visible decay of hypocotyl and toppling over that characterize damped-off seedlings of other pine species are not discernible. Another difference between damping-off of this species and that of most conifers is that the casual fungus or fungi, whose point of infection frequently appears to be at or just below the cotyledon whorl, spread rapidly only in the host tissues at or just beneath the soil surface.

ASSOCIATED FUNGI

Rhizoctonia sp. was the most frequently isolated fungus from diseased longleaf pine seedlings at 3 North Carolina and 1 Mississippi nursery in 1937. Like results were obtained in 1938 at a North Carolina nursery, in 1939 at a South Carolina nursery, and, again, in 1940 at both a North Carolina and a South Carolina nursery. Species of *Fusarium* and *Trichoderma* were occasionally isolated from diseased nursery stock, while *Botrytis* sp. was most frequently isolated in greenhouse tests. Inoculation tests were not made. It is of some interest that species of *Pythium* were not among the fungi isolated despite the fact that the technique used should have yielded it.

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[1] Davis, William C., George Y. Young, Dennis H. Latham, and Carl Hartley. Diseases of conifers in forest nurseries. (Div. For. Path., Bur. of Pl. Ind., in cooperation with Civ. Cons. Corps, U. S. Dept. Agr.) (Mimeographed, not numbered) 63 pp. 1938.

GENERAL OBSERVATIONS

Losses resulting from damping-off of longleaf pine are known to have been more serious in seedbeds sown with 2-year-old, or older, seed than in those sown with the current year's seed. For example, at one nursery contiguous seedbeds were sown on the same day with current year's and 2-year-old seed. Loss from damping-off of seedlings was negligible in the seedbeds sown with the current year's seed, but in those sown with the 2-year-old seed it was so severe as to necessitate their reseeding. The following season, 1940, contiguous seedbeds were sown with current year's collection of longleaf pine seed and with the remainder of the seed lot that had given good final stands the preceding season. Both lots of seed gave good emergence, but damping-off again was so severe in the seedbeds sown with the old seed that they were plowed under, while, in the seedbeds sown with new seed, it was not very serious.

It has long been recognized that, for most pines, damping-off losses generally diminish with increase in age of the host until they are quite negligible by the time woody tissues have developed, usually about 4 to 6 weeks after emergence. In contrast, losses resulting from damping-off of longleaf pine sometimes are as great in 4-month-old seedlings as during emergence. This hazard, however, is quite inconsequential where second-year seedlings or transplants are concerned.

Although Hartley and Pierce,[2] in 1917, recognized that conifer nurseries located on acid soils were less subject to damping-off than those on neutral or alkaline sites, yet the relationship of the soil pH to the damping-off of longleaf pine has not been established. The association of heavy losses in this species with an application of lime made shortly before seeding at a South Carolina nursery, located on acid soil, leads to the inference that the damping-off hazard for this species, as for most other conifers, may be greatest on neutral to alkaline soils. Experience of the last 5 years, however, has shown that such losses may also be serious on soils with a pH value of 4.5, and, in a few cases, have occurred on soils with a pH value as low as 3.8.

Drill sowing, generally found more favorable to damping-off of conifers than broadcast sowing,[2, 3] has appeared to bear the same relationship to damping-off of longleaf pine at 5 southeastern nurseries during the last several years. Another factor linked to conifer damping-off, recognized by Hartley[4] and others for some years at northern nurseries, was a generally smaller amount of damping-off in fall-sown than in spring-sown seedbeds. The writer's observations at several southern nurseries indicate that the same is true for longleaf pine.

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[2] Hartley, Carl and Roy G. Pierce. The control of damping-off of coniferous seedlings. U. S. Dept. Agr. Bull. 453. 32 pp. 1917.

[3] Tillotson, C. R. Nursery practice on the national forests. U. S. Dept. Agr. Bull. 479. 86 pp. 1917.

General observations also indicate that the use of sawdust as a seed cover may reduce the severity of losses resulting from damping-off, but it is believed that at some places either fresh or non-hardwood sawdust may prove unsatisfactory as a control method. At a nursery where longleaf pine seed was drill-sown and covered with soil, an estimated 40 per cent of the emerged seedlings damped-off in 1937. The following year at this nursery the seeding was done in shallow, uncovered drills wherein the seed was subsequently covered with pine sawdust 2 or more years old; the damping-off losses that year were estimated at not more than 3 per cent of the seedlings. At another nursery broadcasting of the longleaf pine seed, firming it in the soil, and then covering with old sawdust in the seasons of 1935, 1936, and 1937 were associated with negligible damping-off losses, while serious losses occurred during 1938 and 1939 when the seed was broadcast, firmed in the soil, and covered with soil. The difference in losses in these nurseries cannot be definitely attributed to the methods of covering the seed as all sowing within any one season followed either one or the other practice. More positive evidence that seed-cover practices are related to incidence of damping-off was obtained at yet another nursery. In 1937, at this nursery, broadcast-sown longleaf-pine seed, covered with soil, suffered an estimated 30 per cent damping-off loss, while the estimated loss in the seedbeds where the seed was covered with sawdust was only 10 to 15 per cent of emerged seedlings. The following year similar seeding practices yielded like results. Longleaf pine seedlings arising from seed covered with $\frac{1}{2}$ to $\frac{3}{4}$ inch of sawdust have been observed to suffer greater losses from damping-off than when a cover of only $\frac{1}{4}$ inch of sawdust was used.

Cultivation, weeding, and sheet erosion also appear to be important predisposing factors to damping-off of longleaf pine. Seedlings of this species, in drill rows at 2 nurseries, have been observed more severely attacked in seedbeds where the mechanical cultivator was so adjusted as to ridge the soil at the base of the seedlings than in those where the cultivator was so manipulated as to avoid heaping the soil around the seedlings. During the past 5 years at 1 nursery, and for the past 3 years at 2 others, loosening of the surface soil in young seedling stands by pulling weeds, particularly those with extensive root systems, was followed by an increase in damping-off losses. At yet another nursery watering practices that reduced the splashing of sand onto the seedlings was associated with lighter losses. The partial burying of the seedlings is most frequently encountered in the lower parts of the seedbeds and is most prevalent on sandy soils. This soil drowning of the crowns undoubtedly reduces the natural aeration, and it seems probable that it increases the temperature, of the covered tissues, especially in dark soils. This increased temperature might result in heat lesions or in the temporary lowering of resistance. However, it is probably more important in favoring the disease through bringing soil-borne fungi in immediate contact with the more succulent tissues of the seedling. Regardless of the way the injury is brought about, it appears fairly certain that the covering of the needle bases or partial burying of the seedlings with soil may be generally anticipated to increase the damping-off hazard of this species. There have been similar observations for native spruce seedlings, which have but short needle-free stems.

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[4] Hartley, Carl. Damping-off in forest nurseries. U. S. Dept. Agr. Bull. 934. 99 pp. 1921.

CONTROL TESTS[5]

At many nurseries ordinary coniferous damping-off may be satisfactorily controlled by the use of soil-acidifying treatments applied on the surface immediately following seeding or by disinfecting the soil with such chemicals as formaldehyde or acetic acid some days prior to seeding. However, such treatments, particularly when the disease is active after the seedlings are 6 weeks old or older, have been somewhat unsatisfactory for reducing losses resulting from damping-off of longleaf pine. A search was initiated in 1937 for an adequate control of damping-off of longleaf pine. A preliminary greenhouse pot test, involving 5 replications, each consisting of 1 nontreated pot and 5 with different amounts of ferrous sulphate solutions applied on the surface of the soil immediately after seeding, was made on a sandy-clay loam that had an initial pH value of 6.6. Data from this test showed that the heavier rates of treatment with ferrous sulphate were directly correlated with increased acidity of the top ½ inch of soil; 28 days after treatment the heaviest rate (2 oz. per sq. ft. of surface area) gave soil pH values of 3.8–4.0. There was no indication of delayed or reduced emergence or of injury to the germinating seed or seedlings by any of the various rates of treatment. Damping-off losses at the end of the first 6 weeks were 28 per cent for pots receiving the heaviest rate of ferrous sulphate and 73 per cent for nontreated pots. However, by the time the seedlings were 6½ months old the early advantage was largely nullified by severe late losses, 92 per cent of the emerged seedlings in nontreated pots and 86 per cent of those in pots treated at the heaviest rate having damped off. Slight to no advantage, however, was found for the lighter rates of treatment.

Nursery results, associated with applications of water solutions of ferrous sulphate to the surface of the seedbeds immediately following seeding, have been variable. In a nursery with initial soil pH values varying slightly around 5.0, applications of ½ and 1 avoirdupois ounce of ferrous sulphate to a pint of water per square foot of soil surface, each rate replicated 5 times, failed to reduce damping-off losses that in nontreated areas killed, that year, more than 50 per cent of the longleaf pine seedlings. In another nursery whose initial soil pH value centered around 5.6, plots were treated with 3 rates of ferrous sulphate, each rate replicated 3 times in drill-sown seedbeds and once in a broadcast-sown seedbed in which the seed was covered with old sawdust prior to making the treatment. A gradual decrease in damping-off losses was associated with increased rates of application. An 8 per cent loss occurred on nontreated plots, while seedlings in plots treated at the rate of $\frac{3}{8}$ ounce per square foot suffered no loss. In general, the loss on the broadcast-sown seedbed, where the seed was covered with sawdust, was somewhat less than on the drill-sown ones.

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[5] People who are interested in the problems discussed in this article, but who are not experienced in handling poisonous chemicals, should be careful to secure skilled advice and supervision before using treatments hereinafter outlined.

The damping-off control efficiency of commercial ortho-phosphoric acid in several dilution rates, each replicated 3 times, also was tested at the last-mentioned nursery. Longleaf pine seedlings in areas treated with $\frac{1}{8}$ fluid oz. per sq. ft. of seedbed area suffered but 2 per cent damping-off loss as contrasted with 8 per cent in nontreated areas. A comparison of damping-off data on the various ferrous sulphate-treated areas with those for plots receiving an amount of ortho-phosphoric acid calculated to give an equivalent hydrogen-ion activity in the upper $\frac{1}{2}$ inch of soil, shows the former chemical somewhat more effective than the latter in reducing losses. A treatment combining the ferrous sulphate ($\frac{3}{8}$ avoir. oz. per sq. ft.) and ortho-phosphoric acid ($\frac{1}{8}$ fluid oz. per sq. ft.) also was associated with a damping-off loss of only 2 per cent. If subsequent experience substantiates this preliminary indication of effectiveness of the combination treatment, its use, as compared with that of either chemical alone, will merit consideration, as it would be less apt to contribute to mineral deficiencies in the soil.

Seven replications of plots treated with $\frac{3}{8}$ fluid oz. of commercial formaldehyde to $\frac{1}{2}$ pt. of water per sq. ft. were established on 3 soil types at one nursery. Seven of the total number of plots were treated 3 days prior to seeding and like numbers 4 and 5 days before seeding. Damping-off losses in treated plots were as great as and in most plots greater than in nontreated ones. As these losses for the most part occurred after the seedlings were 4 weeks old it is possible that such treatments would prove advantageous in checking mortality in younger stock. There was no consistent evidence that the time interval elapsing between treatment and seeding date might be related to the incidence of disease; nor was there any indication that the treatment influenced either the rate of emergence or total number of emerged seedlings.

In one nursery, plots of 2-month-old longleaf pine seedlings were sprayed with 2–3–50 and 4–4–50 Bordeaux and 1 lb. of Cuprocide (96.5 per cent cuprous oxide) to 100 gal. of water; a second application of the respective sprays was made 20 days later. Data from 4 replications of each of these treatments failed to give any significant indication of a reduction of late losses from damping-off by these sprays. However, similar tests in this nursery, with Semesan applied with a sprinkling can at the rate of 1/10 avoir. oz. to $\frac{3}{4}$ pt. of water per sq. ft. of seedbed area, were associated with damping-off losses of not more than 10 per cent, whereas the losses in nontreated areas were 50 per cent or more. The following year similar use of Semesan in this and another nursery was associated with marked reduction in damping-off losses. In using this treatment it was found necessary to make the applications either late in the afternoon or on cloudy days in order to avoid "burning" of the seedlings.

Intensive tests of various seeding and cultivation practices in relation to damping-off losses also were made in 2 nurseries. The use of a ¼-in.-deep pine-needle mulch in 7 replications of plots was associated with some reduction in losses. Most promising results, however, were obtained by placing the seed in uncovered shallow rows and subsequently covering them to a depth of ¼ in. with old sawdust. Fourteen sample strips taken at 10-pace intervals on two 416-ft. rows, and 8 two-row plots each 8 ft. long sown in this way, suffered 25 per cent less loss than occurred in nearby seedbeds in which the seed was drill-sown and covered with soil. These data are in agreement with the previously mentioned general observation that the use of old sawdust as a seed cover appeared to be associated with lower damping-off loss. Working the soil away from the base of longleaf-pine seedlings at frequent intervals in 4 plots in a nursery located on sandy soil was associated with near absence of the disease, which was responsible for losses of 50 per cent and more of the emerged seedlings in adjacent areas. It is believed that careful selection of seed, proper seeding, and correct-cultural practices may afford a more practical control than treatment of the soil with chemicals.

SUMMARY

The persistent rosette habit of longleaf pine seedlings leads to unusual damping-off symptoms. *Rhizoctonia* sp. has been most frequently isolated from diseased seedlings, but inoculation tests have not been made. Species of *Pythium* have not been isolated.

The control problem is distinct in that the period of susceptibility is considerably lengthened by the rosette habit. Tests indicate that a presowing treatment of the soil with formaldehyde probably will not materially reduce late losses nor will spraying the seedlings with Bordeaux or Cuprocide. The use of ferrous sulphate and ortho-phosphoric acid, separately or in combination at time of seeding, reduced losses in some nurseries. Most consistent chemical control of the disease was obtained by sprinkling the seedlings with Semesan. This treatment should be made in late afternoon or on cool or cloudy days in order to avoid "burning." In most nurseries it seems likely that use of highly viable seed, combined with sowing in shallow rows, wherein the seed is covered to a depth of about ¼ in. with sawdust (preferably old), or broadcast-sowing the seed and firming it in the soil and subsequently covering it with sawdust, will afford the most practical methods of control. Further it appears that added protection from the longleaf-pine damping-off hazard would be obtained by the adoption of weeding and cultivation practices that tend to keep the soil away from the bases of the seedlings.

CIVILIAN CONSERVATION CORPS IN COOPERATION WITH
DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

INJURY TO TOMATOES BY LIGHTNING

OTIS C. WHIPPLE[1]

(Accepted for publication February 14, 1941)

INTRODUCTION

Lightning injury to field-grown tomatoes (*Lycopersicon esculentum* Mill.) occurs frequently during electrical storms in southeastern Wisconsin and northern Illinois. Within the past 6 years, injury in 8 fields has been observed by the writer, and specimens from 4 others have been diagnosed. Although the number of fields involved and the extent of damage in each are small, the subject is of interest, since the nature of the injury is unique and often difficult to diagnose. Certain symptoms of lightning effects on tomato have been described previously.[2,3,4,5] Others are recorded here because of their diagnostic value. On one occasion the injury was observed a few hours after the lightning had struck. This afforded an opportunity to study the primary symptoms before the effects of secondary factors had become evident and to follow subsequent developments. Observations have been made on plants at different stages of growth from 6 weeks after transplanting to the time when mature fruit are present.

Circumstantial evidence that the injury herein described was attributable to lightning was obtained in two instances. On August 22, 1936, an electrical storm accompanied by rain arose quickly while the writer was inspecting a field of tomatoes. As he started to drive away, a flash of lightning was observed simultaneously with the sound of thunder in that part of the field he had just left. When the field was visited 4 days later, an area of injured plants was found in the same location. Many of the leaves on the badly injured plants were still green, indicating that the destructive agent was not only recent but rapid in its action. The other incident took place on the night of July 25, 1938, when a severe storm accompanied by much lightning passed over the Chicago area. When the writer visited the Cook County Experiment Station at Des Plaines, near Chicago, the following day, he was shown an area of prostrate tomato plants in an experimental plot. The superintendent, who had observed the injury an hour earlier, stated that it had developed during the preceding 24 hours, and this fact was substantiated further by the appearance of the affected plants.

DEVELOPMENT OF SYMPTOMS

When a strong electrical potential is discharged at a given place in a tomato field, the plants in the immediate vicinity are killed or injured in a

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[1] Deceased, February 13, 1941.

[2] Jones, L. R., and W. W. Gilbert. Lightning injury to herbaceous plants. Phytopath. 8: 270–282. 1918.

[3] Rankin, W. H. Lightning injury of field-grown tomatoes. Canada Exp. Farms Interim Rpt. Dominion Bot. 1920–21: 92–93. 1921.

[4] Brown, H. D., and M. W. Gardner. Lightning injury to tomatoes. Phytopath. 13: 147. 1923.

[5] Hawkins, Stacy. Some effects of lightning on tomato plants. Proc. Indiana Acad. Sci. 42 (1932): 57–59. 1933.

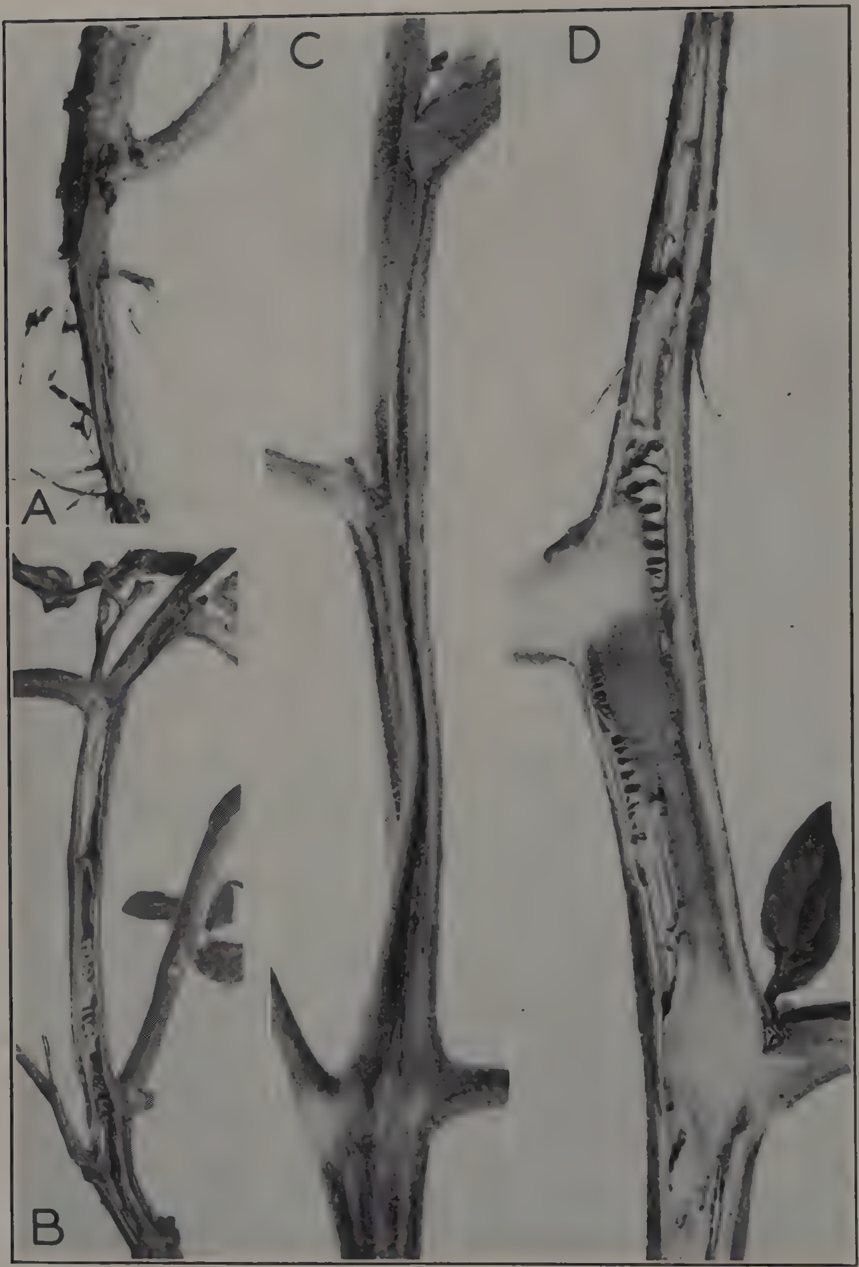

FIG. 1. Lightning injury to stems of tomato. A. Lower portion of a young plant subjected to lightning injury. When the plant was pulled, only the central cylinder of the subterranean stem and lateral roots were removed, leaving the cooked cortical tissue in the soil. B. Portion of a young stem split longitudinally to show extensive destruction of pith. C. A collapsed section of a large branch, showing the flat, ribbed characteristic. D. A large branch cut longitudinally to show the destruction of localized areas of pith or "pith pockets" in the lower and central portions and complete destruction of the pith or "hollow stem" in the upper portion.

circular to elliptical area. Such areas have been found to vary in size from 30 ft. in diameter to 50 × 60 ft. in elliptical areas. The shape of the last type of spot has no relation to the direction of the tomato rows. When lightning strikes a field of young plants, a larger percentage is killed than in fields of mature plants. In one case 57 young plants were killed, whereas 6 was the highest number found dead in any field of mature plants. The larger surface of mature plants and the numerous points of contact between plant and soil may explain this difference. Injury has been found to occur in fields of several soil types and no relation to soil contour seems to exist. Occasionally, the electric discharge causes an eruption in the soil near the center of the damaged area, as previously described by Hawkins.[6] In one case a small jagged hole about 1½ in. in diameter and 4 to 6 in. deep was found about the base of a young tomato plant, leaving it with no anchorage.

The general effect of lightning is an immediate collapse of the parts affected, causing severely injured plants to lie prostrate on the ground as if trampled by an animal. Plants near the center of the area involved are usually killed and the severity of injury decreases in nearly direct proportion to the distance from that point. On plants a short distance from the center, the top branches are most frequently affected, and these either droop or lie prostrate on unaffected parts. At the periphery of the "lightning spot," injury may be confined to a single leaf or growing tip, which usually is on the side adjacent to the damaged area. Some young plants have been found with injury confined to parts near the ground instead of those near the top.

The collapsed stem or petiole appears flattened and ribbed[6] as if subjected to extreme external pressure (Fig. 1, C). The extent of collapse varies with the severity of the injury and may be confined to a few inches, usually near the tip, or it may extend the full length of the stem. When the stem or petiole becomes flattened, it loses its rigidity and, consequently, droops or lies prostrate.

Several types of symptoms result from injury to the cortex. Among these is a streaking of the stem, which may extend through one or more internodes. Irregular areas that vary from a superficial bronzing of the epidermal cells to slightly sunken necrotic areas appear alone or with the streaking of the stem. Still another type of injury to the epidermis and cortex appears in the form of bleached areas suggesting that the tissue had been heated to a sufficient temperature to cook it. These areas vary in size and position, but are especially abundant on the stem[7] of young plants below the soil surface. In many cases the entire cortex of the subterranean stem and roots showed this type of injury, and when these plants were pulled only the central cylinder of the stem and roots were removed, leaving the cortical tissue in the ground (Fig. 1, A). The cortical tissue is usually soft, of mealy texture, and becomes slimy and infested with microorganisms within a few days. Some variations occur in that the tissue may be dry, shrivelled, and even charred. It is probable that all cells are killed in those portions of the stem subjected to high temperatures, but, as stated by Hawkins,[8] the vascular bundles show little injury and, in some cases, may continue to function for some time, as shown by the turgidity of the foliage above the injury.

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[6] See footnote 5.

[7] When tomato plants are transplanted, the tap root ceases development and the new root system consists primarily of lateral roots, which arise from the stem below the surface of the soil. The fields observed were set with transplants.

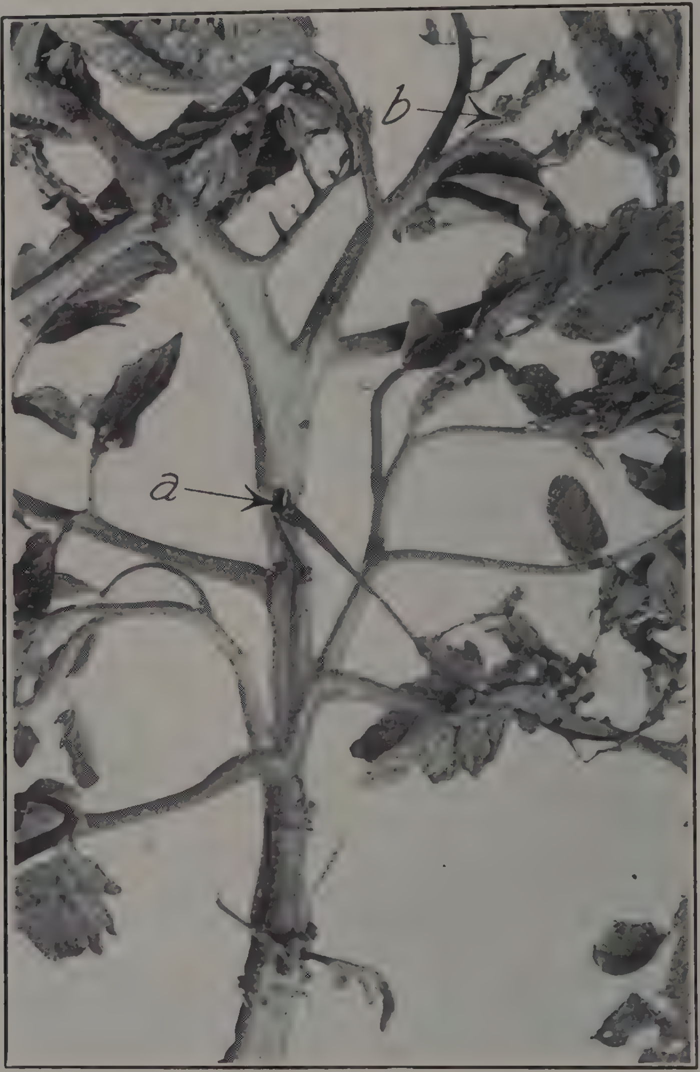

FIG. 2. A portion of a 6-week-old tomato transplant showing severe lightning injury. The hole in the axil of the collapsed petiole at *a* extends to the pith cavity. The passage of the electrical current was violent enough to cause a collapse of the stem at this point. The growing tip at *b* was killed.

Longitudinal fissures and small holes are frequently formed in the stem by the electrical discharge. The lateral crevices vary from $\frac{1}{2}$ in. to the full length of an internode. The depth of these slit-like openings may be limited to the cortex, but they usually extend to the pith. Small holes, which also extend to the pith, are often formed in the axils of the leaves. A typical lesion of this type is shown in figure 2. In this case an electrical charge apparently passed through a leaf midway up the plant with such force as to kill the former, create the hole in the axil, and cause a collapse of the stem at that point. The axillary bud was completely destroyed.

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[8] See footnote 5.

The passage of the charge through the pith invariably results in its collapse and in the formation of a hollow region surrounded by desiccated tissue. The destruction may be confined to small areas or "pith pockets" (Fig. 1, D), or it may extend to form a "hollow stem" (Fig. 1, B), as described by Brown and Gardner.[9] The destruction of the pith always accompanies the collapse of the stem and usually extends for some distance beyond. In severely injured young plants it is often found in the subterranean stem.

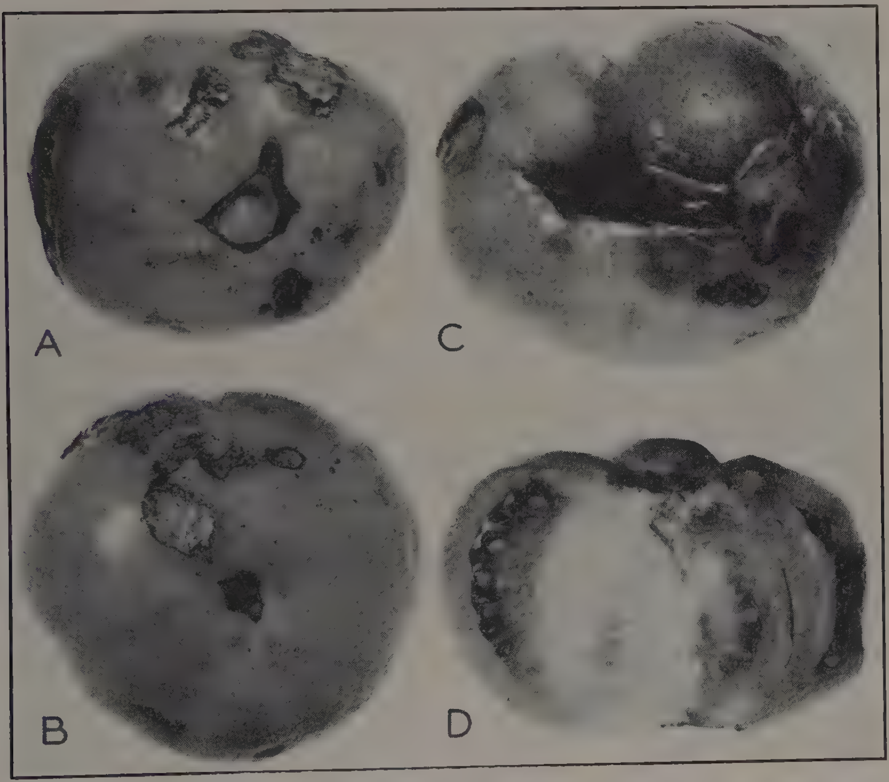

FIG. 3. Mature green fruits injured by lightning. A and B. Irregular burnt or desiccated lesions on portions of fruit nearest the soil; numerous pin-point lesions also are shown in B. C. A large surface blister on the upper right lobe of a fruit, which developed as a result of high temperature. D. The same fruit as in C, cut longitudinally through the blister to show the partially cooked, shrivelled condition of the interior.

The collapse of the petiole is the most conspicuous injury to the leaf, but the leaflets may also show irregular, scalded or burnt areas, and in some cases the entire leaf is completely desiccated or charred. Affected leaves disintegrate rapidly in humid atmosphere.

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[9] See footnote 4.

Fruits on affected plants also show injuries that vary from irregular burnt areas to complete scalding and blistering of the surface. Injury is most common in fruit on or near the ground, indicating a transfer of the charge between the fruit and the soil. In figure 3 typical injury to 3 mature green fruits is shown. Fruits in figure 3, A and B, show the irregular, burnt or desiccated areas nearest the soil. Numerous pin-point necrotic areas may be seen near the blossom end of the fruit in A, which was in contact with the soil. Both are slightly shrivelled, indicating that they were subjected to a lethal temperature. In figure 3, C, a mature green fruit with a large blister is shown. The same fruit, cut through the blister and shown in figure 3, D, exhibits the partly cooked condition. Completely cooked fruits were commonly noted. In fruits some distance above the ground, injury consisted of small, irregular, sunken burnt areas, always within a few centimeters of a large stem, indicating that the charge had jumped the short distance between fruit and stem. In one area the force of the discharge was so violent that a number of the green fruits dropped because of breaks at the pedicel or loosening of the torus.

The growth response of survivors in one field of young plants and in 2 fields of mature plants was observed. Numerous branches from the axillary buds below the injured part of the stem developed, giving the plant a bushy appearance. Growth was retarded and the few fruits set did not ripen. No new growth developed on the injured portion of the stem and the terminal bud was usually killed. In addition to the numerous suckers below the injured section of the stem, Hawkins[10] also reported the development of short branches above the injury, giving the plant the appearance of an inverted feather duster.

SUMMARY

Lightning injury unlike most diseases, parasitic or nonparasitic, results in immediate death or collapse of the parts of the plant affected; secondary changes follow rapidly. Since fields are not often visited for several days following an electrical storm, plants that are killed may be of little diagnostic value, except that they show a prostrate condition. Plants mildly affected usually show certain characteristic symptoms. Among the most conspicuous signs are: (1) collapse of the stem and drooping of the tops; (2) various degrees of hollowing of the stem pith; (3) collapse and desiccation of individual leaves of plants near the periphery of the "lightning spot"; (4) small longitudinal or circular stem lesions; (5) irregular burnt areas on stems, leaves, and fruits; (6) blistering of the surface of the fruit and various degrees of cooking of the underlying tissue.

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[10] See footnote 5.

EFFECT OF FERTILIZER MATERIALS AND SOIL AMENDMENTS ON DEVELPOMENT OF APOTHECIA OF SCLEROTINIA FRUCTICOLA

KARL BAUR AND GLENN A. HUBER[1]

(Accepted for publication February 11, 1941)

Plant pathologists are showing an increased interest in the control of plant diseases by reduction of the "survival level" of the pathogen. Efforts have been made to accomplish this by cultural practices, the use of eradicant fungicides, and other means. The potentialities of this approach to disease control, together with a review of pertinent literature, is presented in a recent publication by Keitt and Palmiter (7). The problem is admirably stated by them in their discussion of apple scab: "The current method of scab control by protecting fungicides is, therefore, based chiefly on the philosophy of defense. The pathogen is permitted to maintain a high survival level, and under the conditions of moderate or severe occurrence of disease there is usually little or no cumulative control."

In the control of brown rot (*Sclerotinia fructicola* (Wint.) Rehm) of prunes in western Washington main reliance has been placed on the use of protective fungicides. Even with the most effective of these practices, brown rot has caused losses of 8 to 10 per cent, in contrast to much higher losses when no control measures were attempted. Under current control practices no effort is made to reduce the survival level of the pathogen by the destruction of the apothecia, which have been found to be the principal source of primary inoculum in the spring (6). The prevention of the development of apothecia or their destruction prior to ascospore discharge should materially reduce the spore load in the orchards in the spring and thus lessen the infection of blossom parts, shedders, and ripening fruit. Cultural practices, often recommended for the destruction of apothecia, have been found impractical for western Washington conditions (4).

A preliminary report on the use of calcium cyanamid for the destruction of apothecia has been published (4). This paper presents further information on the use of calcium cyanamid, as well as other fertilizer materials and soil amendments in the control of apothecia, and includes a discussion on methods of application.

EXPERIMENTS WITH FERTILIZER MATERIALS AND SOIL AMENDMENTS

The principal requirements of a suitable material to destroy apothecia are: (1) the retention of its toxicity sufficiently long to destroy all apothecia that emerge (under western Washington conditions apothecia emerge over a period of from 2 to 3 weeks); (2) it should not alter the soil reaction materially or have a cumulative effect, so that continuous application might prove

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[1] The writers are indebted to Drs. L. C. Wheeting and F. D. Heald for helpful suggestions in the preparation of the manuscript.

injurious; (3) it should lend itself to rapid application, since it is necessary to cover a large area in a short period at the time of first emergence of apothecia; and (4) the use of the chemical should be practical on the basis of cost.

The efficiency of fertilizer materials when applied in both dry form and aqueous solutions were tested as a means of control of apothecia. Each aqueous solution[2] was applied to triplicate plots either with a hand sprayer or with a sprinkling can. Ammonium sulphate, sodium nitrate and urea were applied at rates that would supply approximately 69 lb. of actual nitrogen per acre. The results of these applications on development of apothecia are presented in table 1. No further work was carried on with aqueous solutions of fertilizer salts, for, although definitely toxic to apothecia that had already emerged, they did not prevent later emergence. Furthermore, experience in Clark County has shown that it was impractical and, in some cases, impossible to handle portable spray equipment in orchards in the spring because the soils were not firm enough to support the weight of the machinery.

TABLE 1.—*Results of applications of aqueous solutions of fertilizer materials applied March 29, 1940, on development of apothecia*

Materials	Pounds per acre	Apothecial counts, 36 sq. ft. (April 1)
$CO(NH_2)_2$	150	35
$NaNO_3$	432	25
$(NH_4)_2SO_4$	324	28
Check	....	33

Several fertilizer materials and soil amendments were applied in dry form to determine their effectiveness in apothecial control. Known quantities of the materials were placed into the hopper of a small power duster and the flow so regulated that the experimental areas could be covered at least three times to insure good distribution. All applications were made in triplicate when the first apothecia appeared in the orchard.

Although slight reduction in population of apothecia was obtained from the application of certain materials, the data in table 2 show that, with the exception of calcium cyanamid, no complete control of apothecia was obtained.

It was found that granular or crystalline materials were of little value in apothecial control, as it was impossible to cover the entire surface of the soil completely, especially when small quantities of materials were used.

Although Ezekiel (3) showed that little or no apothecial development occurred in sand cultures at a pH value approaching neutrality or slight alkalinity, it does not appear feasible to add calcium to the soils in the prune areas of Western Washington in sufficient quantities to maintain the pH value at a level necessary to prevent the development of apothecia. Since

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[2] One lb. of material to 5 gal. of water.

TABLE 2.—*Results of application of dry fertilizer materials and soil amendments made April 5, 1939, on development of apothecia*

Materials	Rate per acre	Average number apothecia per 64 sq. ft. (Apr. 13, 1940)
	lb.	
Check	....	12
$CaCN_2$[a]	200	0
$CaCN_2$	300	0
$CaCN_2$	400	0
$Ca(OH)_2$	500	4
$Ca(OH)_2$	1000	8
$CaCO_3$	675	23
$CaCO_3$	1350	10
$(NH_4)_2SO_4$	300	2
$(NH_4)_2SO_4$	500	6

[a] Commercial pulverized and oiled (5 per cent).

the calcium materials were not applied until after the first apothecia appeared and were not incorporated with the soil, it does not appear likely that any appreciable change in the pH value of the soil occurred.

Almost complete destruction of apothecia is necessary to reduce the spore load to a level sufficiently low to be effective in brown-rot control. Of the materials used, only calcium cyanamid has fulfilled these requirements, hence it was the only material tested in later studies.

EXPERIMENTS WITH CALCIUM CYANAMID

As reported in previous papers (1, 4), pulverized and oiled (5 per cent) calcium cyanamid appeared to offer the best possibilities for the destruction of apothecia because of its prolonged period of toxicity. Data presented in table 3 give further proof of the effectiveness of this material.

TABLE 3.—*Apothecial counts on treated and nontreated plots in orchards in Clark County, Washington. Duplicate areas of 36 sq. ft. were examined, April 13, 1939*

Orchard	Treatment	Pounds per acre	Date applied	Average number of apothecia[a]
Firestone (Fruit Valley)	Calcium cyanamid	300	March 20	0
	Calcium cyanamid	300	March 27	0
	No treatment	....	....	17
Moody (Felida)	Calcium cyanamid	300	March 20	0
	Calcium cyanamid	300	March 27	0
	No treatment	....	....	21

[a] Average 1.95 apothecia per mummy.

Since calcium cyanamid has proved effective in reducing the number of prune thrips, *Taeniothrips inconsequens*, which emerge from the soil (2, 5), an effort was made in the spring of 1940 to so time the treatment as to reduce the emergence of thrips, as well as to prevent apothecial development. In

1940 thrips began to emerge in large enough numbers on March 12 and 13 to justify treatment, one week prior to emergence of the first apothecia, March 20.

On March 13, the surface of the soil and vegetative cover of two orchards in the Fruit Valley district, designated as orchards 1 and 2, respectively, were dusted with calcium cyanamid at the rate of 277 lb. per acre. Duplicate blocks containing 4 trees each and covering in each orchard an area of 1600 sq. ft. were left as nontreated checks. On March 14, another orchard in the Felida district, designated as orchard 3, was dusted with the same material, at the same rate per acre. A power machine, described below, was used to make the applications.

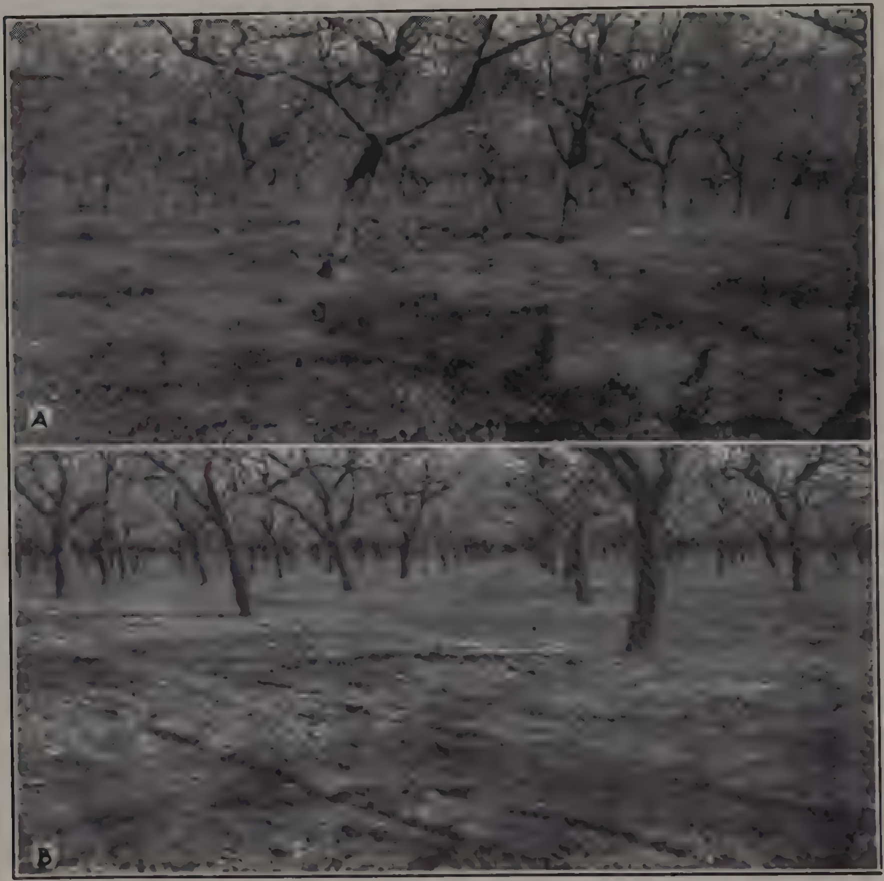

FIG. 1. A. Nontreated portion of orchard showing vegetative cover. B. Portion of orchard 7 days after treatment with calcium cyanamid at rate of 277 pounds per acre.

As a result of the treatments the vegetative cover (Fig. 1) in these orchards was burned to the ground. Frequent examinations of the treated and nontreated areas were made. The first apothecia were observed on the nontreated areas March 20. On March 27 triplicate areas of 36 sq. ft. in each plot were examined, the results of which are presented in table 4.

TABLE 4.—*Effect of calcium cyanamid on development of apothecia in Clark County, Washington, 1940. Triplicate areas of 36 sq. ft. were examined in each orchard, March 27*

Orchards	Treatment		Average number apothecia[a]	
	Date	Pounds per acre	Treated areas	Nontreated areas
No. 1 (Fruit Valley)	Mar. 13	277	0	21
No. 2 (Fruit Valley)	Mar. 13	277	0	22
No. 3 (Felida)	Mar. 14	288	0	40.3
No. 4 (Fruit Valley)	Mar. 16	100	0	20.3
		200	0	17.6

[a] Average 2.32 apothecia per mummy.

Apothecia were abundant on nontreated areas, while no apothecia were found on those treated with calcium cyanamid. On April 4, near the end of the period of development, an occasional apothecium was observed in the treated orchards. Upon careful examination of an area of 8000 sq. ft., 10 apothecia were found. Under conditions of heavy rainfall (Fig. 2) following the treatments, calcium cyanamid apparently lost some of its toxicity before the period of apothecial development was over. In orchards treated March 19 primarily for the control of apothecia, no apothecia emerged. In 1939, when only traces of rain fell (Fig. 2) after treatments, applications of calcium cyanamid, made March 20, were as effective as those made March 27, just before emergence of apothecia. The effect of moisture and temperature (Fig. 2) on decomposition of calcium cyanamid has been discussed in an earlier paper (4).

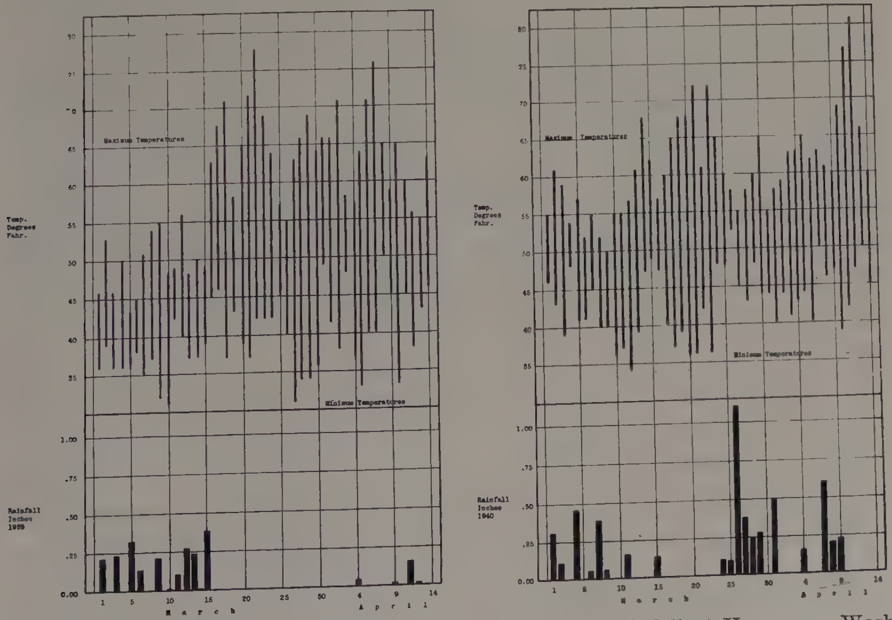

FIG. 2. Maximum and minimum air temperatures and rainfall at Vancouver, Washington, 1939 and 1940.

Under the high rainfall conditions of 1940 a single application of calcium cyanamid was not toxic for a sufficiently long period of time to prevent the emergence of early developing thrips and the later developing apothecia. Under similar conditions in the future it may be desirable to split the application, applying half of the material for thrips control and the other half later, to prevent the emergence of apothecia. Lighter applications of calcium cyanamid when applied March 16 for the control of thrips (2) effectively prevented their emergence and prevented the development of apothecia as effectively as heavier applications (Table 4).

The only apparent effect on the trees from the applications of calcium cyanamid made for the control of brown rot was a general improvement in appearance of foliage similar to that obtained from applications of other nitrogenous fertilizers. The leaves on trees in treated areas were darker green and larger, and they remained on the trees later in the fall than those on trees in nontreated areas. All the plots included in this study were located on Felida silt loam, the characteristics of which have been described (4).

METHODS OF APPLICATION OF CALCIUM CYANAMID

Since calcium cyanamid when applied to the soil has a limited period of toxicity, it is necessary to delay application until thrips or apothecia appear. This delay makes it necessary to apply the materials to large areas in a short period of time. A study of dusters was made, as machines of sufficient volume or capacity to cover areas as rapidly as necessary were not available. After trying several commercial machines, a home-made duster was secured and adapted (Fig. 3). This machine was a narrowed-down automobile, with the motor driving both the machine and the duster. The hopper held 300 lb. of calcium cyanamid. The material dropped directly from the hopper into a 16-in. fan with 4-in. blades revolving horizontally. The dust was blown into a 12-ft. tapered boom[3] with $\frac{3}{8}$-in. holes at 1.5-in. intervals along its lower side. The outer 18-in. section of the boom was elevated at an angle of 45° to provide sufficient overlap in the tree rows for dusting the entire soil surface without cross treatment. In order to prevent the cyanamid from blowing into the trees, a hood 3 ft. wide was constructed and placed over the tapered boom. This consisted simply of a framework of iron pipe and bamboo rods covered by heavy burlap that trailed 5 ft. behind the machine. The heavy bamboo rods were used on the outer 4 ft. of the hood to prevent breakage or bending of pipe when an obstacle, such as a tree, was encountered. The boom was made flexible by using a single short joint of rubber hose 4 ft. from the outer end. This hose was perforated with holes to allow complete coverage of the ground with the calcium cyanamid. A better distribution was obtained with the tapered boom than with the ordinary "vegetable outlets." Since the specific gravity of the material is much greater than the ordinary dusts applied in disease or insect control, it did not spread or "fan" as well through the "vegetable outlets," thus making the application uneven. This machine covered one-half the width between tree rows and its capacity was approximately 3 acres per hour. No injury attributable to calcium cyanamid was found on apricot, peach, or prune blossoms or buds in orchards dusted with the machine described above.

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[3] The boom was 3 inches in diameter at the inlet end and 2 inches in diameter at the outer end.

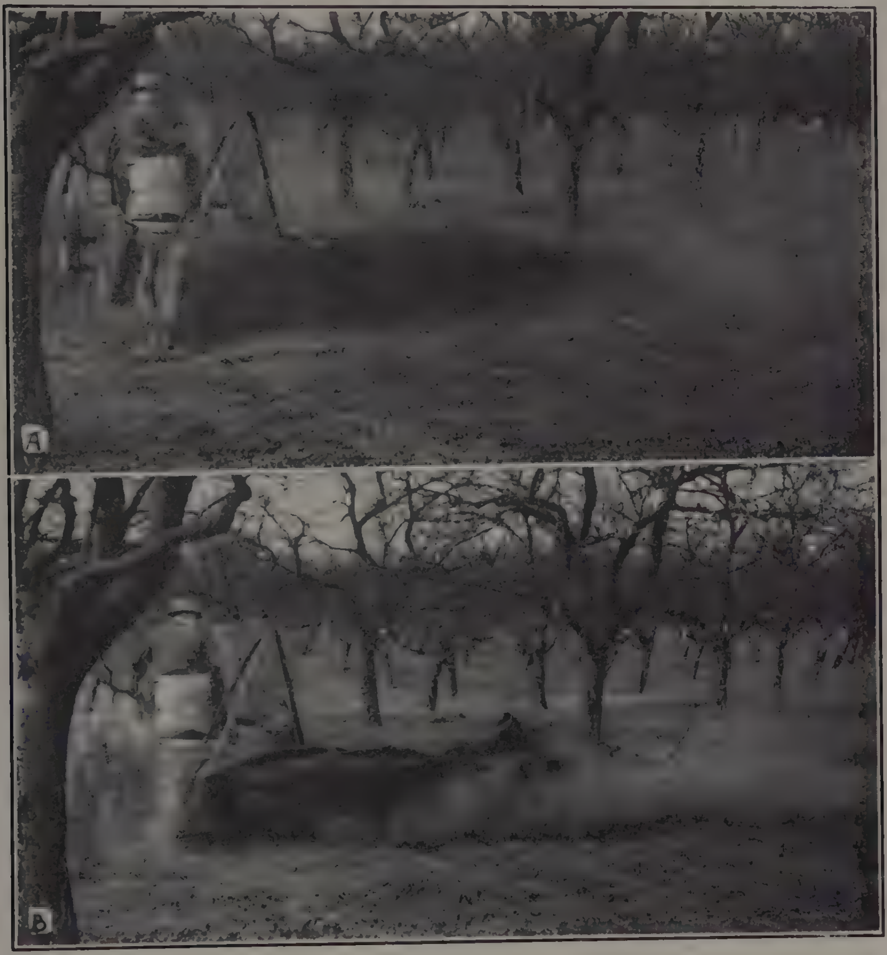

FIG. 3. Duster used in applying calcium cyanamid. A. Hood lowered. B. Hood raised.

SUMMARY

The effects of surface applications of hydrated and carbonated lime and of fertilizer salts to the soil under trees, in both dry form and aqueous solutions, on the development of apothecia are here reported. Aqueous solutions of 3 fertilizer salts proved ineffective; of the dry materials used, only calcium cyanamid prevented the development of apothecia.

Calcium cyanamid remained toxic longer when the soils remained relatively dry after application than when the material was exposed to heavy

rainfall. By delaying applications until just prior to apothecial emergence the material remained toxic sufficiently long to prevent development of apothecia.

No toxic effects were observed on trees in plots treated with calcium cyanamid.

The duster described in this paper was especially designed for the application of calcium cyanamid.

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LITERATURE CITED

1. BAUR, KARL, and GLENN A. HUBER. The use of calcium cyanamid and other fertilizer materials and soil amendments in the destruction of apothecia of *Sclerotinia fructicola,* with methods of application. (Abstract) Phytopath. **30**: 785. 1940.
2. BREAKEY, E. P., GLENN A. HUBER, and KARL BAUR. The use of calcium cyanamid in the control of pear thrips, *Taeniothrips inconsequens* Uzel in prune orchards. Jour. Econ. Ent. **33**: 950–951. 1940.
3. EZEKIEL, WALTER N. Hydrogen-ion concentration and the development of *Sclerotinia* apothecia. Science **58**: 166. 1923.
4. HUBER, GLENN A., and KARL BAUR. The use of calcium cyanamid for the destruction of apothecia of *Sclerotinia fructicola.* Phytopath. **29**: 436–441. 1939.
5. ————, ————, and EDWARD P. BREAKEY. The effect of calcium cyanamid on development of apothecia of *Sclerotinia fructicola* and on population of *Taeniothrips inconsequens* in prune orchards. (Abstract) Phytopath. **29**: 825. 1939.
6. ————, and ————. Brown rot on stone fruits in Western Washington. Phytopath. **31**: 718–731. 1941.
7. KEITT, G. W., and D. H. PALMITER. Potentialities of eradicant fungicides for combating apple scab and some other plant diseases. Jour. Agr. Res. [U. S.] **55**: 397–437. 1937.

A LEAF SPOT OF PEAS (PISUM SP.) CAUSED BY CERCOSPORA LATHYRINA[1]

J. L. WEIMER

(Accepted for publication March 1, 1940)

INTRODUCTION

Early in September, 1938, and each summer since a leaf spot has appeared on Austrian Winter and other field and hybrid peas (*Pisum arvense* L. and *P. arvense* × *P. sativum* L.) growing under a cheese cloth shelter at Experiment, Georgia. This disease has never been seen in the field and is not known to be of any economic importance. The fact that it is potentially capable of doing considerable damage to the pea plant and that no record of the occurrence of such a disease in continental United States has been found, makes it seem worthwhile recording briefly the results of the observations and studies made.

SYMPTOMS

The lesions caused by this fungus vary greatly in number, presumably depending on conditions of air temperature and humidity, and are scattered irregularly over the surface of the leaflets (Fig. 1, A). As many as 90 spots have been counted on a leaflet measuring 50 by 30 mm. and 15 lesions have been seen on 1 sq. cm. of leaf surface. The lesions are separate, or rarely confluent, and the dead tissue is not excised. Affected leaves are not killed unless the number of spots is large. The lesions are circular to angular in outline and involve the smaller veins but are restricted by the larger ones. Lesions usually vary from 1 to 5 mm. in diameter, but are sometimes as much as 10 mm. in maximum diameter. They have approximately the same appearance on both sides of the leaflets (Fig. 1, B). The smaller spots are more or less uniform in color, varying from cinnamon-brown[2] to mars-brown to warm-sepia. More commonly, however, the color fades so that the center of the lesion is gray to nearly white. The margin remains a shade of brown and may be slightly raised. This brown margin is sharply delimited from the adjacent green tissue, although there is some yellowing when the larger veins have been killed or the spots are numerous. There may be a second concentric ring near the center of certain lesions immediately surrounding the gray area (Fig. 1, A).

The pathogen may attack the young stems, petioles, and tendrils, although on the latter the lesions are not numerous. These differ little from those on the leaves, except that their longest diameter is parallel to the axis of the part attacked. Tendrils may be girdled and killed, but no serious damage resulting from stem infection has been observed.

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[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 81 Journal Series, Georgia Agricultural Experiment Station.

[2] Ridgway, Robert. Color standards and color nomenclature. 43 pp., 53 color plates. (Washington). 1912.

ETIOLOGY

An examination of the lesions showed the presence of a *Cercospora.* Isolations were made, and inoculations demonstrated that this fungus was responsible for the disease. The organism grows well on ordinary agars, but fruits little if at all on any media tried.

Infected pea leaves, held out of doors over winter, were placed in a moist chamber for 2 days at laboratory temperature early in March, and a new crop of spores typical of the *Cercospora* were produced in the center of the old lesions. It is apparent that the fungus remains alive over winter in the old lesions, but no evidence has been found indicating that it forms a perfect stage.

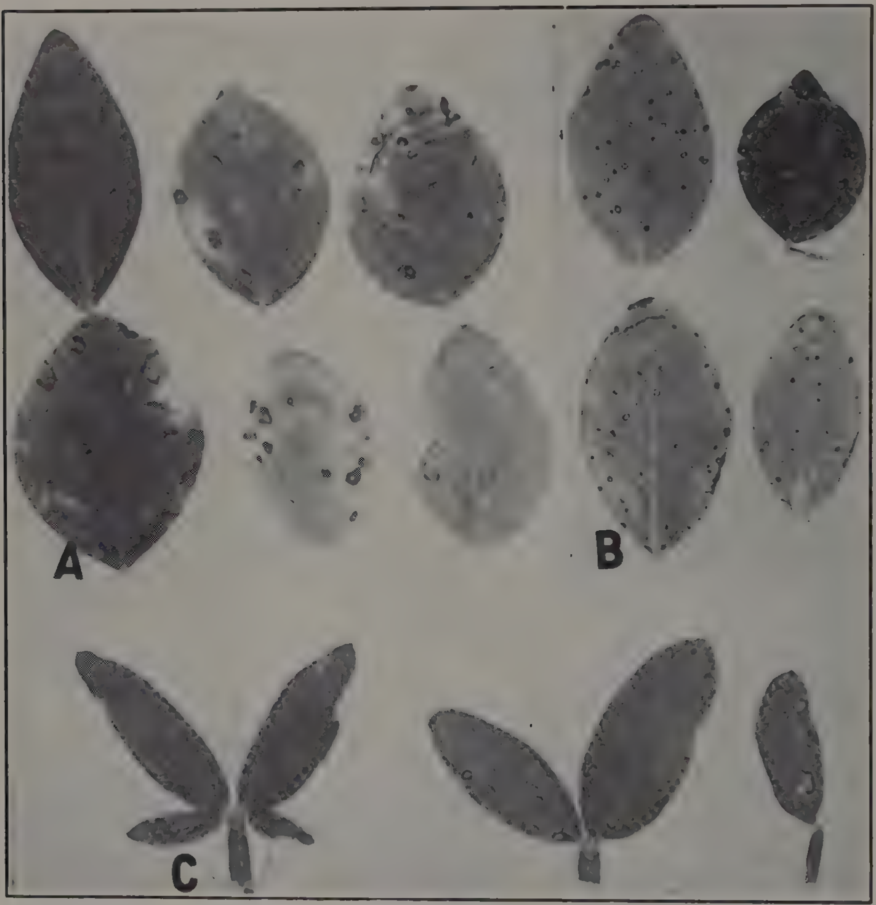

FIG. 1. A. Cercospora leaf spot of hybrid peas (*Pisum arvense* × *P. sativum*) caused by *Cercospora lathyrina.* Leaflet at the upper left is healthy, the remainder are diseased. In some cases the tissue outside of the lesions has turned yellow, as denoted by the lighter color, while in other instances no such change is apparent. Note the darker brown margin and in many lesions the small brown ring near the center. × approximately 1. B. Leaf spot on hybrid pea leaflets. The lesions have approximately the same appearance on the upper (two top leaflets) and under (two lower leaflets) sides. Somewhat reduced. C. Leaf spot on perennial sweet pea (*Lathyrus latifolius* L.). Somewhat enlarged.

It is evident that the fungus spreads under conditions of low rainfall, since new lesions continued to appear on new growth during an extremely dry period in October and November, 1939, when there was only 0.13 and 0.68 inch, respectively, of precipitation. Some difference in susceptibility of different hybrids was noted, although none was immune.

The species of *Cercospora* causing this disease was at first in doubt. Similarity of the lesions caused by this fungus and those caused by *C. pisa-sativae* Stevenson indicated that the two diseases might be the same. However, a comparison of Stevenson's[3] fungus with the one under investigation showed some differences. *C. pisa-sativae* is described as having conidia measuring 3–4 × 50–240 μ, averaging 130 μ long. Spores of the writer's fungus measure 3.2–5.7 × 25–325 μ, averaging 3.82 × 87.5 μ. It was found that the spores varied greatly in length, depending on the conditions under which they were produced. Most of those studied were from the host, where they had developed under very dry conditions. Spores formed under such conditions seldom reached 100 μ long. However, those formed on infected leaves held in a moist chamber for 2 or 3 days measured from 100 to 325 μ long. It was observed that the width of the spores remained quite constant regardless of the conditions under which they developed (mostly 3.6 to 4.2 μ). It is apparent that spore length is not a dependable taxonomic character of this fungus, but spore width is. The conidiophores of the 2 species measure respectively 4.5–6 × 50–170 and 4–4.8 × 35–90 μ.

Specimens were submitted to J. A. Stevenson who informed the writer that the lesions on the leaves from Georgia and Puerto Rico were very similar as to size, and marginal characteristics, but that the Puerto Rican fungus spreads out from the original spot and fruits freely over much of the leaflet, in decided contrast to the behavior of the Georgia fungus, which fruits sparingly and only in the center of the lesions. He stated also that the conidia from the Puerto Rican material were considerably larger than those from Georgia. The specimens sent to him had been taken from the cloth house, where they had been formed under dry conditions, hence they were small.

Specimens were sent also to Charles Chupp at Cornell University, who kindly compared the Georgia material with co-type specimens of *Cercospora pisa-sativae* and *Cercosporas* on other closely related hosts and concluded that the species under consideration in this paper is *C. lathyrina* E. and E. He also compared a *Cercospora* from perennial sweet pea (*Lathyrus latifolius* L.) (Fig. 1, C), the host of *C. lathyrina*, from Griffin, Georgia, and found this to be identical with that on peas.

A single attempt was made to confirm the conclusion that the pea and perennial sweet pea *Cercosporas* were the same species. Spores scraped from naturally infected pea leaves were placed in water and sprayed on both pea and perennial sweet-pea leaves and the plants kept moist. A few typical lesions developed on a few of the perennial sweet-pea leaflets, while those of the pea were quite heavily infected. Isolations from naturally infected leaves from both hosts gave apparently identical fungi.

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[3] Stevenson, J. A. Report of the Division of Plant Pathology and Botany. Ann. Rept. Insular Exp. Stat., Porto Rico (1917–1918): 138. 1919.

SUMMARY

A leaf spot of peas (*Pisum* sp.) caused by *Cercospora lathyrina* E. and E. is reported and described. This disease is caused by the same fungus as that causing a leaf spot of perennial sweet pea. It has been seen only at Experiment, Georgia, on peas growing under a cloth shelter during late summer and early autumn.

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SILVER NITRATE AS A STAIN FOR USE IN STUDIES OF CONDUCTION OF LIQUIDS IN WOOD[1]

JOHN W. JOHNSON

(Accepted for publication February 17, 1941)

INTRODUCTION

During the course of studies dealing with the California flatheaded borer (*Melanophila californica* Van Dyke), which were conducted in northeastern California in 1939 by the Bureau of Entomology and Plant Quarantine, the effect of scars of flathead larval galleries upon conduction of liquids through the wood of ponderosa pine (*Pinus ponderosa* Lawson) was given considerable attention. In order to secure information on this phase of the problem, a method of staining green wood tissues was sought that would demonstrate and preserve clearly the pattern of primary longitudinal liquid movements in wood, uncomplicated by lateral diffusion from the primary channels.

A survey was made of methods and stains used by other workers in studies of conduction and sap flow in plants. However, the techniques referred to seemed not to satisfy fully the requirements of the present problem. To meet these requirements, it seemed necessary, therefore, to make a study of stains and staining methods. A staining technique was needed that would (a) demonstrate the conduction pattern as it existed during the primary movement of a staining solution longitudinally through the wood, and (b) preserve the pattern of staining in the wood for later analysis.

PRELIMINARY TESTS

The first tests were made with filtered saturated solutions of the aniline stains scarlet red, water green, and methylene blue. Two methods of applying the stain solutions to the blocks of green wood were tested. The first consisted of placing the lower ends of the wood blocks in vessels of staining solution, capillary action being depended upon to carry the stain through the wood. It was found that capillary force failed to bring about an active stain movement through the wood blocks. In the second method pressure was used, and the stain solutions were injected into the wood blocks through a rubber tubing fitted over their ends. Pressure injection resulted in a rapid flow of the staining solution longitudinally through the wood. It, therefore, was used for all later tests.

When alcoholic aniline stains were applied under pressure for a few minutes and the sample was split open immediately afterward, a definite pattern of staining was observable. Such a pattern presumably was representative of the course of the stain as it existed when the solution was dynamically moving longitudinally through the sample. It was observed, however, that, in a few minutes following injection, the stain spread laterally into unstained areas, thus obscuring the initial pattern and making its analysis impossible. It seemed necessary, therefore, to find a staining solution that could be fixed in the wood immediately after injection, so that the conduction pattern, representative of the longitudinal movement of the stain, could be preserved and lateral movement, due to the high diffusibility of the solution could be prevented.

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[1] In carrying out the research on this problem, much assistance and many valuable suggestions have been given to the writer by his associates in the Bureau of Entomology and Plant Quarantine. Their aid and the helpful comments of other workers during the preparation of the manuscript, are gratefully acknowledged.

Consideration of other solutions that might fulfill these requirements resulted in the selection of silver nitrate. It has the following desirable features: (a) It forms a true solution, which does not obstruct the wood tracheids and can be applied as readily as aniline stains; (b) it is easily soluble in alcoholic concentrations as high as 20 per cent; and (c) the silver is readily precipitated upon the wood, thus offering possibilities of fixing the silver on the wood before lateral diffusion obscures the initial pattern. When tests were made with silver nitrate solutions, it was found that this substance met the needs of the problem, and a 2½ per cent silver nitrate in 20 per cent ethyl alcohol solution was selected for further work.

PROCEDURE

The procedure was simple. Wood samples with and without larval gallery scars were cut from the surface of freshly felled green trees. It was not possible to carry out the preparation of samples under water, inasmuch as some phases of the work could not be accomplished with the inadequate field laboratory equipment available. However, the wood was kept wet by frequent applications of water at all times during the process of preparation. Until all samples were ready for staining, the prepared samples were kept fresh by storing them in water chilled to about 32° F. Tests of check samples stored in this way for periods as long as 72 hours after the tree was cut showed no change in conductivity. Each sample was made 3 inches long and 5/16 inch square. The ends were cut across with a sharp razor blade, and the corners of one end were rounded and smoothed to facilitate close application of the walls of the rubber tubing when it was slipped over that end of the sample. In figure 1 is shown a wood sample and the slab of green pine wood from which it was removed.

The silver nitrate solution was stored in an elevated reservoir in the top of the field laboratory and was siphoned out through the rubber tubing that was used for injecting the wood samples. The pressure at the lower end of the tubing was about ¼ of an atmosphere. A clamp on the lower end of the tubing controlled the flow of the stain.

When the tubing was slipped over the end of the sample, a small bubble of air usually was trapped in the tubing against the end of the sample. It was necessary to remove this air so that it would not block the wood tracheids when the clamp was released and pressure applied to the sample. The air was allowed to escape along the side of the wood sample, the clamp being released slightly, so that the solution would force the air from the tubing.

In view of the consistency of the patterns in series of samples that were stained, it seems probable that little blocking due to air occurred. After removal of the air, the solution was applied for 2 minutes, sufficient time to accomplish adequate injection of the sample.

Immediately after application of the solution, the sample was removed from the tubing, split longitudinally, and placed in an elonhydroquinone developer (D 72, Eastman Kodak Company). After 30 seconds in the developer, during which the silver was precipitated out of solution upon the exposed surfaces, the pieces of sample were transferred to acid hypo. This was allowed to act on the surfaces for 1 minute, when the pieces were transferred immediately to a water bath. A longer time in the hypo seemed unnecessary. The samples were washed in flowing water for 10 minutes, giving the silver nitrate remaining in the interior of the wood opportunity to diffuse out and flow away with the water without precipitating on the surfaces. The pieces were then removed from the water, blotted, and dried.

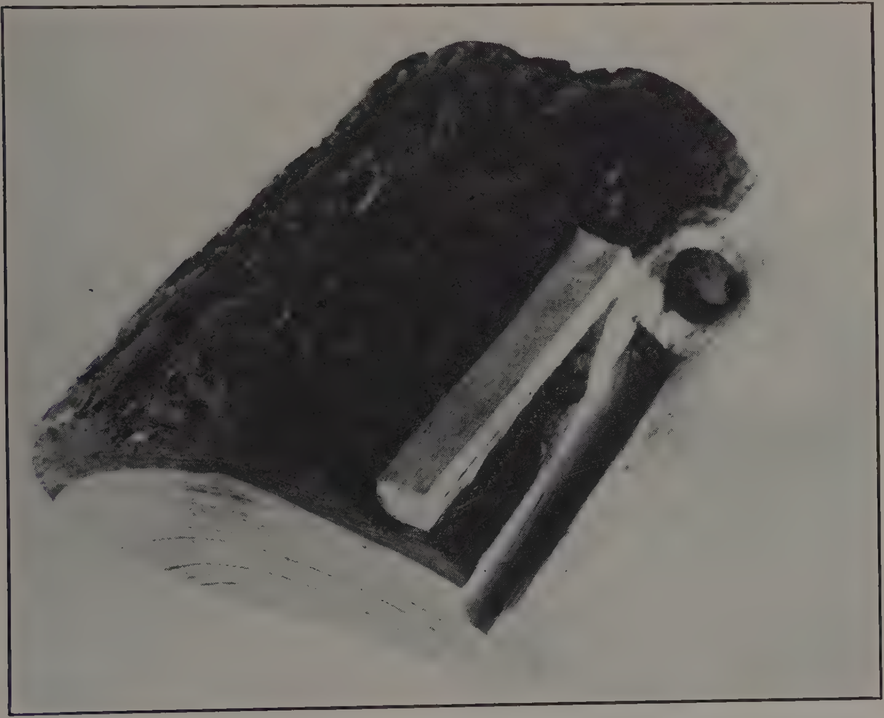

FIG. 1. A green ponderosa pine slab and a wood sample removed from the outer sapwood of the slab preparatory to staining the sample with silver nitrate. Photograph No. 11190C by K. A. Salman. × about 0.8.

DISCUSSION

Precipitation of the silver on the surfaces of the split samples appeared to preserve the pattern of conduction in the condition obtaining at the time of splitting of the sample after its injection. The washing of the samples in water further prevented obscuring of the pattern produced on the split surfaces by carrying away silver nitrate that subsequently diffused out of the interior of the wood. It was possible by this technique to preserve clearly the pattern of the course of the liquid, presumably as it existed when the stain was flowing through the wood. Boundaries between stained and nonstained areas were sharp. The preparation of the solution was easy, and the equipment could be easily obtained and set up. The appearance of the wood samples after treatment is illustrated in figure 2, A and B.

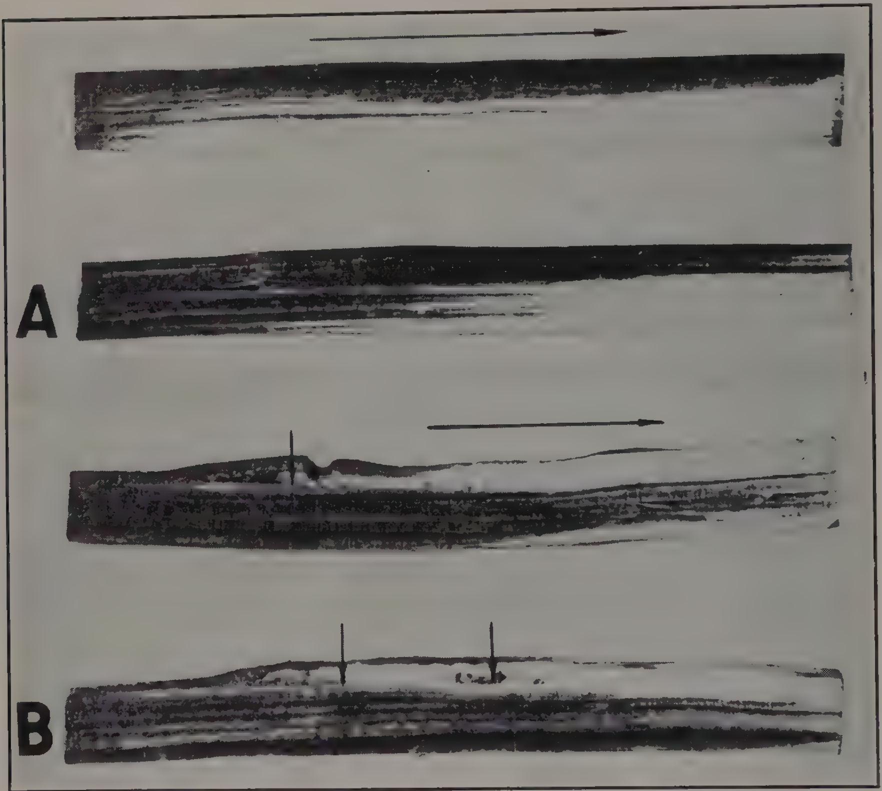

FIG. 2. Sections from split samples unscarred (A) and scarred (B) ponderosa pine sapwood after injection with silver nitrate and development of the silver. The dark staining (silver deposits) indicates the pattern of liquid conduction. Direction of movement of the solution is shown by the arrow. The upper edge of each sample is the outer cambium surface of the wood. Note that in B the silver nitrate did not reach the wood beyond the scars, which are indicated by the smaller arrows. Photographs No. 11199 by J. E. Patterson. × about 1.3.

The final effect is purely a surface phenomenon. Only the surfaces of the split sample are brought into contact with the developer, as its penetration into the wood is slow. The pattern represents the conditions of stain conduction along a particular plane in which the samples were split. It would be desirable to find a means of precipitating the silver in place throughout the wood, quickly, without allowing time for lateral diffusion. The possibility of injecting the developer into the wood immediately after the application of silver nitrate was considered, thus effecting precipitation of silver within the wood sample; but this has not yet been tried.

The method as here described was developed for a particular purpose, with the limited facilities and equipment of the field laboratory as were available. Its application under laboratory conditions may reveal its limitations, or result in refinements, and demonstrate its usefulness in other problems.

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THE VAPOR PRESSURE GRADIENT ABOVE A TRANSPIRING LEAF

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It hardly seems necessary to dwell on the important role that microclimates, as it were, adjacent to leaf surfaces, frequently play in spore germination and in events that occur during the incubation period, as well as in some fungus developments and in insect ecology. The results reported by Longrée,[1] to cite one specific and pertinent example, were essentially that the spores of the powdery mildew of roses require a high relative humidity (97–99 per cent) for germination, and that the presence of water in the liquid state is definitely detrimental to germination. The germination on rose leaves is good, even though the vapor pressure in the air at some distance from the plants is relatively low. The implication is that the microclimate adjacent to the leaf is a moist one, and, as such, is a very important factor in the infection of the plant by the fungus.

It is interesting to speculate about some of the properties of these microclimates. One would naturally expect that the vapor-pressure conditions adjacent to leaf surfaces are not qualitatively different from those adjacent to a free liquid surface where the diffusion process is the factor that tends to equalize the partial vapor pressure throughout the available space. We are not aware of any measurements having been made on the vapor pressure of water at various distances from the free liquid not in equilibrium with its vapor phase, but Ramsey, Butler, and Sang[2] have ingeniously determined the relative humidities at various distances from transpiring leaves in a wind-tunnel under conditions controlled with reference to vapor pressure, rate of air flow, temperature, and illumination. We have made use of their data in this communication.

The differential equation for the free diffusion of a single substance is

$$\frac{\delta c}{\delta t} = D\left(\frac{\delta^2 c}{\delta x^2} + \frac{\delta^2 c}{\delta y^2} + \frac{\delta^2 c}{\delta z^2}\right) \tag{1}$$

where D = diffusion constant
c = concentration
t = time
x, y, and z have their usual meanings

Under circumstances where the diffusion occurs in only one direction, as would be the case in the diffusion of water vapor from an aqueous surface, equation (1) reduces to

$$\frac{\delta c}{\delta t} = D\,\frac{\delta^2 c}{\delta x^2} \tag{2}$$

It frequently occurs that there are extraneous disturbances, such as streaming, convection currents, gravitational force, etc., that influence the diffusion process. Under the simplest of these conditions the equation for diffusion reduces to

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[1] Longrée, Karla. The effect of temperature and relative humidity on the powdery mildew of roses. Cornell Memoir 223: 1–43. 1939.

[2] Ramsey, J. A., C. G. Butler, and J. H. Sang. The humidity gradient at the surface of a transpiring leaf. Jour. Exp. Biol. 25: 255. 1938.

$$\frac{\delta c}{\delta t} = D\frac{\delta^2 c}{\delta x^2} - \frac{\delta(cv)}{\delta x} \tag{3}$$

where v is the normal component of velocity induced by the extraneous disturbance. If conditions prevail such that v is not a function of time, a stationary state will eventually be reached where

$$\frac{\delta c}{\delta t} = 0 = D\frac{\delta^2 c}{\delta x^2} - \frac{\delta(vc)}{\delta x} \tag{4}$$

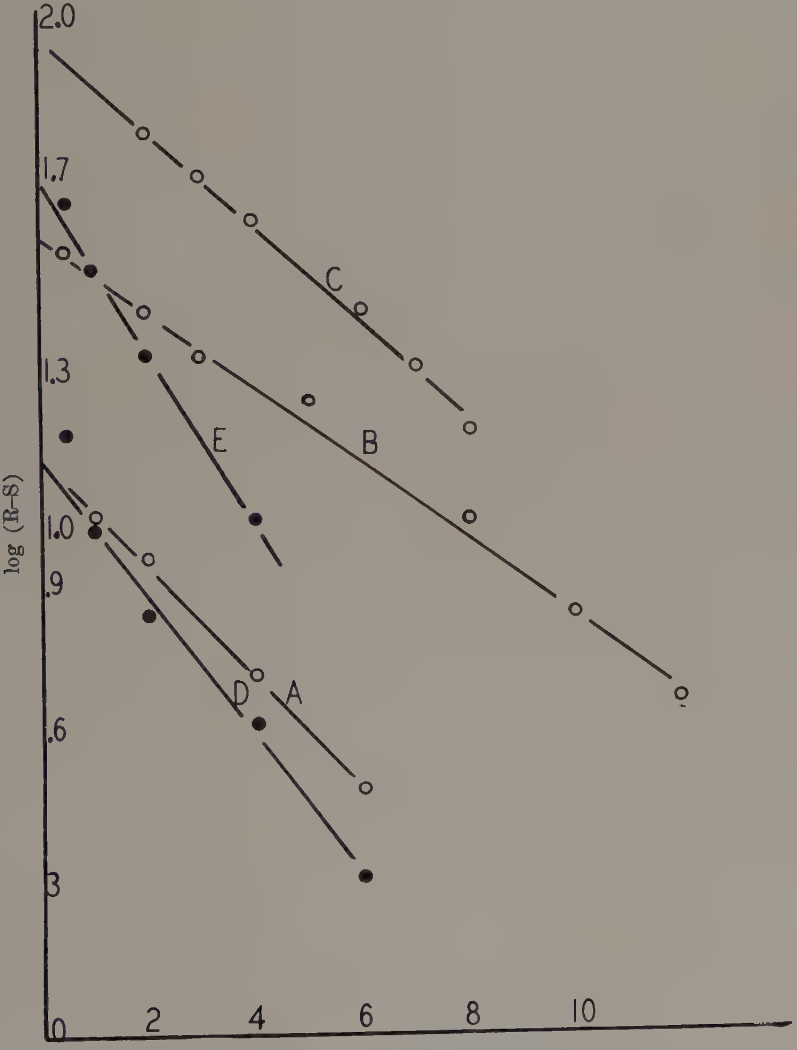

FIG. 1. Values of log (R–S) plotted against distance from the several surfaces studied by Ramsey, Butler, and Sang, where R is rel. humidity at a given distance from surface and S is rel. humidity at long distance from surface.

The solation of equation (4) is

$$\begin{aligned}\text{Log } c &= -\frac{v}{D}x + \text{constant} \\ &= -\frac{v}{D}x + \log N \qquad (5)\end{aligned}$$

where log N is the integration constant, and the value of $-v/D$ will be determined by the extraneous conditions. At the point where $x = 0$ (at the water surface for example) $\log N = \log c$, or $N = c$. Log c is directly proportional to x. The exponential law given in equation (5) holds generally, subject to the restrictions imposed by the experimental set up. In dealing with gaseous diffusion, one may substitute the partial pressure of the gas for concentration in these various equations.

In figure 1 we have plotted the values of log (R–S) against distance from the various surfaces studied by Ramsey, Butler, and Sang, where R is the observed relative humidity at a given distance from the surface, and S is the relative humidity at a large distance from the surface. It will be observed that log $(R-S)$ is proportional to the distance from the leaf. In the case at hand we may substitute the contribution by the leaf to the relative humidity $(R-S)$ for the partial pressure of the water vapor and rewrite equation (5).

$$\log (R-S) - \log M = -\frac{v}{D}x \qquad (6)$$

where M assumes the dimensions of relative humidity.

The substitution is justified by virtue of the fact that the dimensions of c and N (equation 5) must be the same (that of partial pressure of water vapor, if we so choose) and by the fact that c and N each may be multiplied and divided simultaneously by the vapor pressure of water at the temperature in question without changing the equation. Equation (6) demands that log $(R-S)$ be proportional to x; note that observation and theory agree. In the equation $-v/D$ determines the slope of the curve and is influenced by the extraneous conditions; log M is the intercept when $x = 0$, and is probably characteristic for the surface in question. At the surface of the leaf $R = M + S$.

In order to determine the relative humidity at the surface of a leaf, assuming suitable experimental conditions, it is only necessary to determine S at a large distance from the leaf, and R at two arbitrary distances from the leaf.

Curves A and B in figure 1 were obtained from the data of Ramsey, Butler, and Sang for the relative humidity gradient over dock leaves as measured by an evaporimeter; curve C was obtained from their data for the gradient over a wet filter paper; curves D and E are for tulip leaves, as measured by a hygrometer.

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SMUT SORI FROM OVARIAL AND STAMINAL TISSUES OF CERTAIN GRASSES[1]

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In certain smuts of native Kansas grasses belonging to the Andropogoneae it was found that there is a wide range in size and shape of the sori of a given smut species on a particular host. Upon close examination, it was found that these differences were correlated with the organs from the primordia of which the sori were produced. These differences in size of the sori are particularly striking in *Sorosporium everhartii* Ellis and Gall. on *Andropogon furcatus* Muhl.

The spikelets of *Andropogon furcatus* are borne in pairs at each node of an articulate rachis. At each node, one spikelet is sessile and perfect, the other pedicellate and staminate (2). The latter also bear vestigial ovaries. These rudimentary ovaries of the pedicellate spikelets, as well as the ovaries of the fertile sessile spikelets, are often found affected by *Sorosporium everhartii* (Fig. 1, A and B). When the staminal tissues are infected and develop into separate sori, these are smaller and much more slender (Fig. 1, A, h) than those produced from ovarial tissue (Fig. 1, A, d). The studies here reported were made in 1935 on 148 affected spikelets from specimens collected 5 miles southwest of Manhattan, Kansas, (collection No. 1); and on 219 affected spikelets from specimens collected in the U. S. Dept. Agr. Grass Nursery, Kansas State College, Manhattan, Kans., (collection No. 2).

Of all the sori produced from ovarial tissue, 39 per cent, in collection No. 1; and 32 per cent in collection No. 2, were produced in the pedicellate spikelets. That is, these sori developed from rudimentary ovaries, which, normally, never develop seed. The sori from them, however, averaged almost as large (Fig. 1, B, e and n) as those developed from the ovarial tissue of the sessile, normally fertile spikelets (Fig. 1, B, a and j).

In individual spikelets, sessile or pedicellate sori may develop from ovarial tissue only (Fig. 1, B, a, e, j, and n); from ovarial tissue and 1, 2, or 3 staminal primordia (Fig. 1, B, b, f, k, and o; c, g, and i; and d, h, m, and q); or from staminal primordia only (Fig. 1, A, h, and B, p). In collection No. 1, 91 sessile and 57 pedicellate spikelets were studied. Eighteen per cent of the former and 56 per cent of the latter had sori from staminal tissue. Similarly, in collection No. 2, 148 sessile and 71 pedicellate smutted spikelets were studied. Of these, 4 per cent and 13 per cent, respectively, had developed sori from staminal tissue. Thus, it appears that there was a greater tendency for staminal sori to develop in the pedicellate than in sessile spikelets. It is impossible to state whether the higher percentage of staminal sori in the one collection, 5 miles southwest of Manhattan, was because of the fungus, host, environment, or a combination of these features.

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[1] Contribution No. 412 from the Department of Botany, Kans. Agr. Exp. Stat., Manhattan, Kansas.

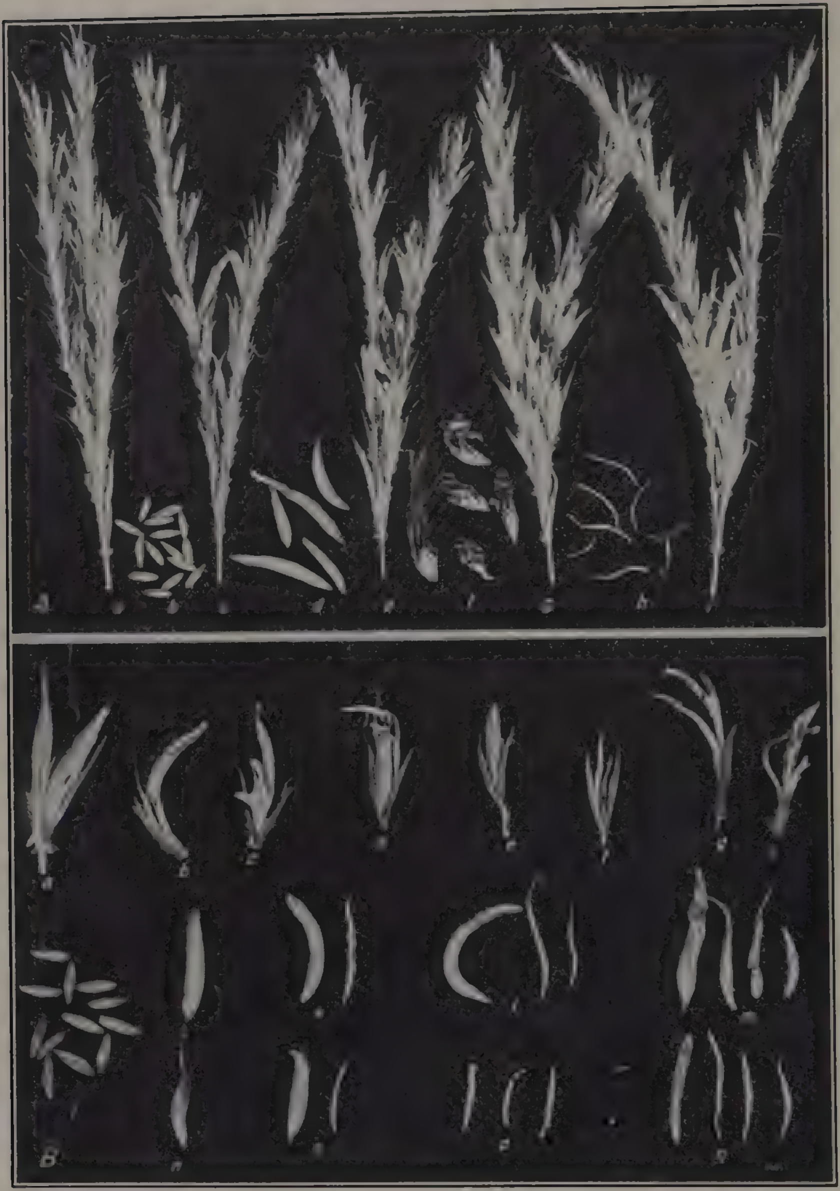

FIG. 1. A. *Sorosporium everhartii* on *Andropogon furcatus*. a, normal inflorescence; b, normal seed; c, e, g, and i, smutted inflorescences. Staminal sori are especially apparent in the central part of the right raceme in e. d, immature ovarial sori; f, mature ovarial sori; h, staminal sori. × ½. B. a, sessile spikelet with one sorus; b, sessile spikelet with two sori; c, sessile spikelet with three sori; d, sessile spikelet with four sori; e, pedicellate spikelet with one sorus; f, pedicellate spikelet with two sori; g, pedicellate spikelet with three sori; h, pedicellate spikelet with four sori; i, normal seed of *Andropogon furcatus;* j, one sorus from a sessile spikelet; k, two sori from a sessile spikelet; l, three sori from a sessile spikelet; m, four sori from a sessile spikelet; n, one sorus from a pedicellate spikelet; o, two sori from a pedicellate spikelet; p, three sori from a pedicellate spikelet; q, four sori from a pedicellate spikelet. × ¾.

Sori from staminal, as well as ovarial tissue, also were found in *Sorghum vulgare* infected with *Sphacelotheca sorghi* (Link) Clinton and *S. cruenta* Potter in the smut nurseries at Kansas State College, Manhattan, Kans., in 1936. In both of these smuts certain sori were found with compound tips, as shown for *S. sorghi* in figure 2, A, in contrast with the more common form of tip shown in figure 2, B. The compound tip is made up of 4 parts, 3 from staminal tissue and 1 from ovarial tissue. These parts are united below to form the usual simple base. Apparently, even the sori with simple tips have originated from both staminal and ovarial tissues and the parts united very early in the development. Some of the otherwise almost smooth tips showed faint marks suggesting compound origin.

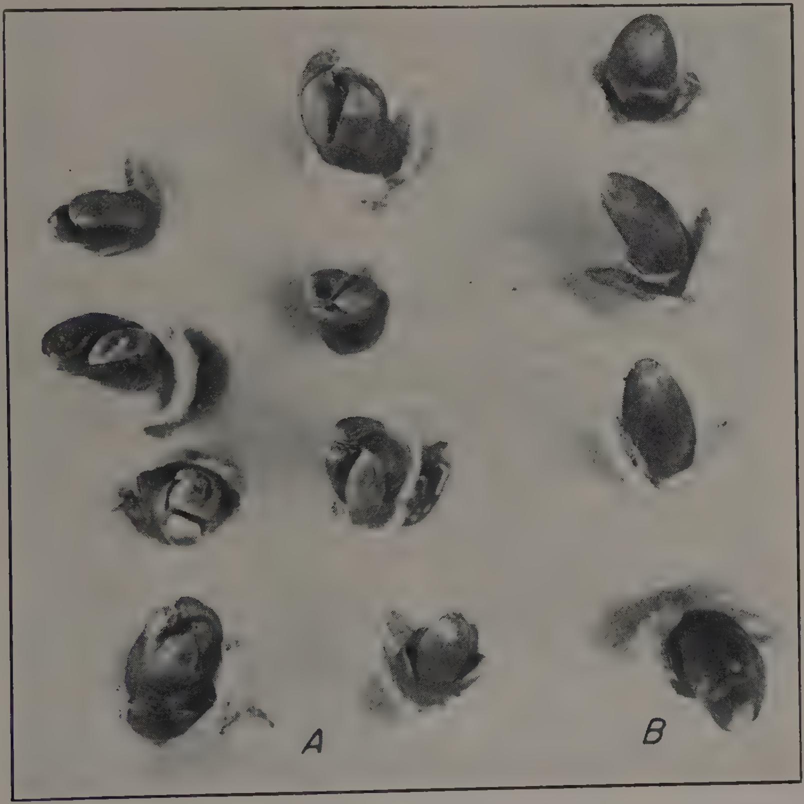

FIG. 2. Sori of *Sphacelotheca sorghi* from *Sorghum vulgare*. A. Sori having compound tips. B. Sori having simple tips. × 3.

Hansing (1) found that 50 per cent of the pistillate sori of *Sphacelotheca occidentalis* (Seym.) Clinton on *Andropogon furcatus* and of *S. cruenta* (Sholci) Jackson on *Sorghum halepense* (L.) Pers. were in the pedicellate spikelets. Rudimentary ovaries of *Buchloë dactyloides* (Nutt.) Engelm. have been found similarly affected by *Tilletia buchloeana* K. and S., as have also those of *A. furcatus* (*A. provincialis* Lam.) and *A. hallii* Hack. by *S. occidentalis* (*Ustilaga andropogonis* K. and S.) (3).

The male inflorescence of *Zea mays* L. is often found affected by *Ustilago zeae* (Beckman) Angen. and *Sorosporium reilianum* (Kühn) McAlpine.

Similar phenomena have been observed in the case of certain smuts of dicotyledonous plants (4 and 5).

SUMMARY

Variations in size and shape of the sori of certain smuts of grasses are discussed and are correlated with the organs from the primordia of which the sori originated. These are characters that may have importance in the identification of grass smuts.

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LITERATURE CITED

1. HANSING, E. D. A preliminary study of certain smuts of native grasses—Andropogoneae. (Unpublished thesis) Kansas State College of Agriculture and Applied Science, p. 1–94, pl. 1–12. 1937.
2. HITCHCOCK, A. S. Manual of the grasses of the United States. U. S. Dept. Agr. Misc. Pub. 200: 1040 pp. 1935.
3. KELLERMAN, W. A., and W. T. SWINGLE. New species of Kansas Fungi. Jour. Myc. 5: 11–14. 1889.
4. MCALPINE, D. The smuts of Australia. Their structure, life history, treatment and classification. pp. 1–228, pl. 1–56. J. Kemp, Government Printer, (Melbourne). 1910.
5. SAVALESCU, TR. Contributions à la connaissance des Ustilaginées de Roumanie. Extras din Analele Institutuluide de Cercetari Agronomice al Romaniei 7: 347–430. 1935.

INTERSTATE COOPERATIVE EXPERIMENTS ON FIELD SPRAYING OF SOUR CHERRIES[1]

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(Accepted for publication March 10, 1941)

For more than 15 years, a group of experiment station workers interested in the problems of fruit growing in the Cumberland-Shenandoah Valley region have met once a year to exchange ideas and experimental results and to improve and unify recommendations made to growers in the 4 States involved. The value of this conference to the workers, at least, has been considerable, and some degree of unity in recommendations has been brought about. In recent years, the prosecution of carefully planned cooperative experiments has appealed to several members of the group as offering the best basis for securing still greater uniformity in recommendation. This is an account of such an experiment conducted in 3 States during the 1940 season, involving a test of materials for the control of cherry leaf spot (*Coccomyces hiemalis*).

METHODS

The experiment consisted of 13 single-tree plots and a check, randomized and replicated to 6 times within each State. The trees were all of the variety Montmorency and comparable in age, size, and vigor. The proprietary materials entering into the various sprays were supplied to the cooperators by the manufacturers from the same factory batch, thus making certain of their uniformity. The schedule consisted of 4 spray applications, timed uniformly in relation to tree development. The 4 sprays were applied at the following periods: (1) petal-fall, (2) shuck-fall, (3) 3-week-cover, and (4) post-harvest. Uniform methods of collecting data made it possible to pool the results for statistical analysis.

Treatments and Formulae.—**Plot 0,** nonsprayed check; **plot 1,** Bordeaux 2–4–100[2]; **plot 2,** lime-sulphur 1–50; **plot 3,** lime-sulphur 1–50 (Bordeaux 2–4–100 in 4th spray); **plot 4,** Cupro K 3–3–100; **plot 5,** Basi-cop 3–8–100 (zinc sulphate, monohydrate, ½ lb.); **plot 6,** Pheno-thiazine 4–4–100; **plot 7,** tank-mix phosphate (copper sulphate 2 lb., tri-sodium phosphate 2 lb., lime 3 lb.); **plot 8,** "ZO" 3–3–100; **plot 9,** Copper Hydro "408" 3–3–100; **plot 10,** Compound "A" 1½–3–100; **plot 11,** Tennessee "26" 3–3–100; **plot 12,** as No. 11, with 1 pt. Orthex; **plot 13,** as No. 11, with 1 pt. Nu-film (a rosin-residue emulsion).

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[1] Authorized for publication on March 10, 1941, as paper no. 1022 in the Journal Series of the Pennsylvania Agricultural Experiment Station, as Scientific Paper no. 261 of the West Virginia Agricultural Experiment Station, and as Paper no. 109 of the Section of Plant Pathology of the Virginia Agricultural Experiment Station.

[2] All formulae are expressed in pounds per 100 gal. The first figure refers to pounds of copper compound and the second to hydrated lime. Lead arsenate (Orchard Brand) at 2 lb. per 100 gal. was used in the first 3 applications on all plots except the check and No. 6 (Phenothiazine). The lime called for in these formulae was the "Gold Bond" brand of the National Gypsum Company.

Fruit data.—The effect of the spray materials on size of fruit was determined by counting the number of cherries in a 1-lb. random sample of fruit from each tree. In table 1 each figure in the "state" columns thus represents an average of 6 lb., and each figure in the "average" column is an average of 18 lb.

Foliage data.—The effect of the various sprays on the control of leaf spot was determined by leaf counts so arranged as to reflect the amount of defoliation. Prior to fruit harvest 10 branches were selected at random on each tree. These branches were tagged, each with two tags in such a manner that each pair of tags bracketed 50 leaves. This resulted in 500 marked leaves per tree, or 3000 leaves per treatment in each of the 3 States. Subsequent

TABLE 1.—*Effect of different spray materials on size of fruit as determined by the number of cherries per pound random sample from each tree*

Treatment	Pennsylvania	Virginia	West Virginia	Average[a]
Check	94.5	113.0	115.3	107.6
ZO	96.3	123.7	116.3	112.1
Cu Hydro "40"	102.2	114.0	120.3	112.2
Cupro K	106.2	116.3	116.5	113.0
Tennessee "26" and Nu-film	104.0	121.8	114.2	113.3
3 L. S. & 1 Bord.	100.2	121.2	119.3	113.6
Lime Sulphur	101.5	121.0	121.0	114.5
Basi-Cop	103.8	123.3	117.0	114.7
Tennessee "26" & Orthex	100.2	129.0	115.3	114.8
Tennessee "26"	106.3	126.3	120.8	117.8
Compound "A"	105.5	127.2	125.5	119.4
Phenothiazine	105.2	129.8	124.7	119.9
Tank mix Copper Phosphate	112.0	129.8	124.0	121.9
Bordeaux 2–4–100	116.2	127.2	129.5	124.3
	Least significant difference		20: 1	5.95[b]
	" " "		100: 1	7.85

[a] Averages, excepting those affected by missing values, were determined directly from the 18 plot totals.

[b] Applies to averages only.

TABLE 2.—*Effect of spray treatment on number of cherries per pound*

	Analysis of variance		
	Degrees of freedom	Sums of squares	Variance
Blocks[a]	15	2,771.90	184.79
Treatments	13	4,564.16	351.08
States	2	18,053.76	9,026.80
State × treatment	26	1,878.35	72.24
Error	194	15,921.43	82.02
Total	250[b]	43,180.60	....

[a] The degrees of freedom and sum of squares for the blocks were found by addition after determining each State separately. This form of analysis is based on that proposed by Snedecor, G. W., in the third edition of "Statistical Methods," p. 240–242. 1940.

[b] Degrees of freedom are 250 instead of 251 because of the necessity of supplying missing data on one of the check trees.

counts of the leaves on these marked branches were made at intervals following the last spray application and the results expressed as the percentage remaining at any given date. The records made during the first week of October are presented here.

RESULTS

The data in tables 1 and 2 indicate, on both inspection and analysis, that the size of the fruit varied with the State in which it was produced. While this fact can perhaps be explained by variation in rainfall and other climatic factors, it is not pertinent to the present investigation. The data also indicate that nonsprayed trees produced the largest fruit. Since successful cherry culture demands protection against leaf spot, it is necessary to base comparisons for size of fruit upon the standard spray practice and not upon the nonsprayed checks. In this region lime sulphur has been the standard

TABLE 3.—*Percentage leaves remaining first week of October, 1940. Treatments aranged according to the average leaf retention*

Treatment	Pennsylvania	Virginia	West Virginia	Average
Compound A	71.7	74.7	82.2	76.18
Tennessee ''26'' & Nu-film	66.9	71.9	85.2	74.73
Tennessee ''26''	67.0	67.1	83.3	72.47
Cu Hydro ''40''	68.2	64.6	83.2	72.00
Cupro K	65.3	67.7	82.3	71.77
Tank Mix copper phos.	61.9	61.4	79.0	67.43
Tennessee ''26'' & Orthex	66.3	60.7	73.4	66.78
3 L. S. & 1 Bord.	64.9	69.0	65.7	66.53
Basi-Cop	55.1	52.6	88.9	65.50
ZO	48.5	45.0	87.0	60.28
Bordeaux 2–4–100	68.9	51.1	56.8	58.92
Phenothiazine	17.4	10.8	60.8	29.67
Lime sulphur	27.5	21.3	15.3	21.38
Check	1.6	2.8	9.6	4.67
Least significant difference 20 : 1				8.18[a]
" " " 100 : 1				10.80

[a] Applies to averages only.

TABLE 4.—*Effect of spray treatment on percentage leaves remaining. Analysis of variance*[a]

	Degrees of freedom	Sums of squares	Variance
Blocks	15	6,052.84	403.52
Treatments	12	62,263.59	5,118.63
States	2	13,754.29	6,877.14
State × treatment	24	14,457.80	602.40
Error	177	27,390.90	154.75
Total	230[b]	123,919.42	....

[a] Analysis as in table 2.

[b] Degrees of freedom = 230 because the check plots were omitted from the analysis, and it was necessary to supply the figures for three plots destroyed by wind. The missing values were estimated by the method of C. H. Goulden ''Methods of Statistical Analysis,'' p. 261–263. 1939. See also Baten, U. P., Mich. Agric. Expt. Sta. Tech. Bull. 165. 1939.

material. It is, therefore, of interest to find that both tank-mix copper phosphate, and Bordeaux mixture, in the formulae we employed, resulted in fruit significantly smaller than when lime sulphur was used.

In tables 3 and 4 it is again obvious, both from inspection and from the analysis, that the results varied with the State. Actually, the test orchards in both Pennsylvania and Virginia suffered a much earlier outbreak of leaf spot than did the orchard in West Virginia. The differences between treatments are, however, very highly significant and may well form the basis for the elimination of the more unsatisfactory materials from further consideration, even though it is not possible to pick a "best" material with certainty from a single season's results.

The method of determining the amount of defoliation perhaps deserves a further word of comment. Cherry leaves fall for at least two reasons; first, because they are infected by the leaf-spot fungus; and second, because they may be injured by the spray applied. We have not, in this study, attempted to determine the percentage or severity of infection, except by observation. The number of leaves remaining is, therefore, an indication of two characteristics of the fungicide, namely its value as a fungicide and its safety.

DISCUSSION

While many States have reported on spraying experiments with cherries, and our results are, in part, confirmatory rather than new, we are not aware of any previous attempt to pool in a single analysis the results of cooperative tests such as are here reported. Cooperation in research has been emphasized so much in recent years that the suggestion has become anathema to many investigators. We believe, however, that many problems actually lend themselves to the type of cooperative effort here set forth, and, furthermore, that in many instances such effort would result not only in a greater uniformity of control recommendations, but in considerable saving of time. In this instance we find 3 criteria for the elimination of certain materials from further tests. In the first place, we can eliminate Bordeaux mixture and tank-mix copper phosphate in the formulae and schedules used because of their effect on the size of the fruit. In the second place, we can eliminate lime sulphur and phenothiazine because of consistent failure to control leaf spot in the schedule used. Thirdly, certain materials may be eliminated because of their inconsistent effect upon leaf retention. ZO and Basi-Cop gave excellent leaf retention in the West Virginia test where the leaf spot epiphytotic began comparatively late, but did not stand up under the conditions of heavier infection that prevailed in Pennsylvania and Virginia. Had the test been run in West Virginia alone, at least one additional year and perhaps more would have been necessary to make this fact apparent.

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THE PATHOGENICITY OF PAIRED HAPLOID LINES OF USTILAGO ZEAE VERSUS THE PATHOGENICITY OF NUMEROUS MIXED HAPLOIDS[1]

M. F. KERNKAMP AND W. J. MARTIN

(Accepted for publication March 3, 1941)

In the course of studies of *Ustilago zeae,* it has been observed that inoculating corn with a composite mixture of a number of haploid lines causes a different degree of infection than the single haploid pairs that are included in the composite inoculum. Similar results were reported by Holton and Heald[2] and by Rodenhiser and Quisenberry,[3] who found that some varieties of wheat resistant to a composite inoculum of *Tilletia* were highly susceptible to some of the individual collections included in the mixture.

In these experiments Northwestern Dent corn seedlings were inoculated in the greenhouse with single pairs of haploid lines of *Ustilago zeae* and with bulk inoculum comprising all of the haploid lines used in the single pairs. All inoculations were made by injecting potato-dextrose broth suspensions of *Ustilago zeae* into the plants. The results of the first experiment appear in table 1. In recording the results, arbitrary infection ratings were set up on the basis of the number of plants infected and the number and size of galls

TABLE 1.—*The comparison of the degree of pathogenicity of single pairs of haploid lines of Ustilago zeae with that of the composite inoculum*

Inoculum[a]	Infection rating
HaA2–2–1 × HaD4–4	0 fleck
HaC3–5 × B.C.B3–4	0 fleck
HaA3–4 × B.C.A1–5	3 +
HaC2 × B.C.C3–1	1 +
HaB4–3 × HaD4–3	2 –
HaC2 × HaC3–5	2
HaB4–3 × B.C.C3–1	2 +
B.C.C3–1 × B.C.A1–5	3
HaA2–2–1 × HaA3–4	2 –
HaD3 × B.C.A1–5	4 +
HaC3–5 × B.C.A1–5	4
HaD3 × B.C.B3–4	1 –
HaA2–2–1 × B.C.C3–1	1 –
HaA3–4 × HaB4–3	2
Composite of all lines listed above	2 –

[a] The label of a haploid line may be interpreted according to the following: HaA2–2–1 is the first variant arising from the second variant produced by the monosporidial line from cell number 2 of the promycelium of chlamyodospore A from a collection from Hastings, Minnesota. Those indicated B.C. are from Brooklyn Center, Minnesota.

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[1] Paper No. 1895 of the Scientific Journal Series, Minnesota Agr. Exp. Station. Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 65–1–71–140, sponsored by the University of Minnesota, 1940.

[2] Holton, C. S., and F. D. Heald. Studies on the control and other aspects of bunt of wheat. Washington Agr. Exp. Stat. Bull. 339. 1936.

[3] Rodenhiser, H. A., and K. S. Quisenberry. Bunt reaction on same varieties of hard red winter wheat. Jour. Am. Soc. Agron. 30: 484–492. 1938.

formed on the plants. The lowest degree of infection was designated "0 fleck" and the highest was "4." These were further qualified by (+) and (−).

As indicated in the table, the virulence of single pairs ranged from 0 fleck to 4+, whereas the virulence of the composite of all the haploid lines was 2−.

It has been suggested that the dilution of the more virulent races may result in their being masked by other, less virulent, races in the mixture.[2] An experiment, therefore, was made in which measured quantities of inoculum were used in the single pairs and the composite inoculum contained equal proportions of each haploid line. In this experiment, 2 series of inoculations were made. One series consisted of 4 single highly virulent pairs and the composite of these 4 combinations. The other series consisted of 4 pairs ranging from relatively low virulence to high virulence and the composite of those lines. Each combination was triplicated and the experiment was repeated 3 times. The results of this experiment are recorded in table 2.

TABLE 2.—*The comparison of the degree of pathogenicity of single haploid pairs of Ustilago zeae with that of composite inoculum when measured amounts of inoculum were used*

Highly virulent combinations	Infection rating	Pairs whose virulence ranges from low to high	Infection rating
HaA3–4 × B.C.A1–5	4+	HaC3–5 × B.C.B3–4	2
B.C.C3–1 × B.C.A1–5	3+	HaA3–4 × B.C.A1–5	3+
HaD3 × B.C.A1–5	4	HaD3 × B.C.A1–5	4+
HaC3–5 × B.C.A1–5	3	HaD3 × B.C.B3–4	2
Composite of all lines listed above	4	Composite of all lines listed above	3
Composite of all lines in both series	3+	....	......

As indicated in table 2, the virulence of the composite inoculum in each series approached the average virulence of the single pairs in each series. Likwise, the reaction of the composite of all the lines in both series was intermediate between the reactions of the composite of both series. These results indicate that the dilution of lines in bulk inoculum is not the explanation of this phenomenon.

It was also postulated that the presence of a weakly virulent pair in a composite inoculum might limit the development of the highly virulent strains within the composite. Therefore, an experiment was made to test this hypothesis.

Corn seedlings were inoculated with a weakly virulent pair of haploid lines; then the same seedlings were inoculated 3 and 6 days later with a highly virulent pair. This was done with several different lines, each being run in triplicate and repeated twice. In no case did the presence of the weakly virulent pair prevent the development of the highly virulent combination in subsequent inoculations. The opposite of this was also done in which plants inoculated with highly virulent pairs were subsequently inoculated with weakly virulent pairs. The latter results, however, were rather erratic and no definite conclusions could be drawn from them. The results show that the presence of one compatible pair of haploid lines in seedlings will not prevent the development of another in the same seedling, although the presence of compatible pairs of haploid lines that are weakly virulent may limit the development of others that are highly virulent.

It was further considered that there might be some antibiotic effect of one pathogenic strain upon another. An experiment was made in which all of the lines used in the inoculation studies were grown in all possible paired combinations on potato-dextrose agar in duplicate 250-cc. Erlenmeyer flasks. In no case was there any indication of one line having an antagonistic effect on another. This, of course, was done under extremely artificial conditions, and does not by any means prove that different lines are not antagonistic to one another in the plants.

Whatever the explanation may be, these results, which agree with others,[2,3] indicate that care should be taken in classifying resistant varieties on the basis of their reaction to bulk inoculum.

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PHYTOPATHOLOGICAL NOTES

A Fusarium Wilt of Sweet William (*Dianthus barbatus*).[1]—In 1939 a serious disease of sweet william, *Dianthus barbatus* L., showing the characteristics of a fusarium wilt, was encountered by C. M. Tompkins in outdoor plantings in the Colma section of San Mateo County and in Golden Gate Park, San Francisco, California. Cultures prepared from affected plants consistently yielded a fungus that fitted the description of *Fusarium oxysporum* Schlect.,[2] and that has been proved pathogenic in subsequent greenhouse tests. This is the first record in the writer's knowledge of a fusarium vascular wilt of this plant.

Symptoms of the disease appear first as a yellowing of the new growth. The yellowing may involve the entire leaf or only parts of it, occurring in the latter case as large indefinite blotches or streaks. With the inception of the disease, growth becomes much retarded, resulting finally in conspicuous stunting, especially when infection occurs early. As the disease progresses the leaves come to point downward, instead of upward as in a healthy plant, and the edges tend to curl slightly towards the under surface (Fig. 1, B). The leaves do not, as a rule, wither or wrinkle, as is sometimes the case following mechanical or other damage to the stem, but tend rather to become

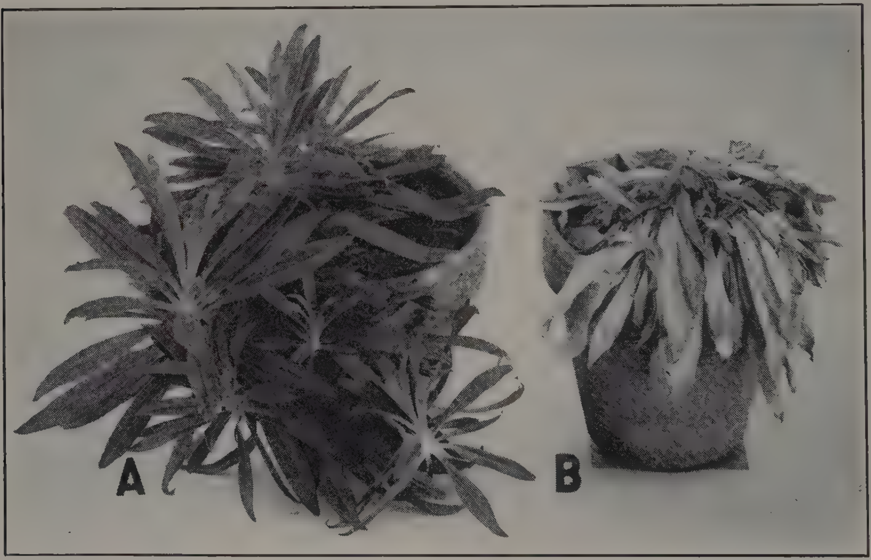

FIG. 1.—Fusarium wilt of sweet william. A. Healthy plant of the same age and treatment as B, but not inoculated. B. Inoculated 6 weeks prior to photographing, by pouring the spore suspension from an agar slant of *Fusarium oxysporum* f. *barbati* n. f., on the soil.

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[1] Assistance in the preparation of this material was furnished by the personnel of Work Projects Administration Official Project No. 65-1-08-91.

[2] Snyder, W. C., and H. N. Hansen. The species concept in *Fusarium*. Amer. Jour. Bot. 27: 64–67. 1940.

leathery. They gradually turn yellow and become tinged with tan as they die. At this stage, yellowing of the diseased plants is in striking contrast to the normal dark green of healthy plants. A brown discoloration of the vascular system of the roots and lower stem attends the foliage symptoms. In large plants a unilateral development of symptoms has been observed. Infection may take place any time from the seedling to, and including, the blossoming stage. As in the case of fusarium wilt of asters, a basal stem rot appears early in the course of the disease and may be considered a secondary symptom caused by the wilt-producing *Fusarium*. The foot-rot symptoms that accompany the vascular wilts of these plants are due in large part, no doubt, to the very close proximity of the vascular elements to the surface of the stem.

Single-conidium cultures of the *Fusarium* isolated from the field specimens were used in all greenhouse inoculation trials, as well as in laboratory studies of the fungus. In one experiment, sterilized soil was infested by incorporating in it chopped-up mats of the fungus grown on potato-dextrose broth, and healthy transplants were placed in it. In later experiments, inoculation was obtained by pouring a spore suspension of the fungus over the soil of 6-inch pots, each containing a healthy plant. Greenhouse soil, infested in 1939 and allowed to stand idle approximately 8 months after the plants in it had died, was still highly infested at the end of this time, as measured by the rapid wilting of healthy plants newly transferred to it. Field evidence indicates that soil once infested remains so indefinitely.

Whether healthy transplants were placed in infested soil, or the fungus introduced into non-infested soil (by means of a spore suspension) supporting healthy plants, symptoms usually developed within 2 to 4 weeks (sometimes within a week). Often severe wilting, even death, had occurred a month or 6 weeks following exposure of the plant to the fungus.

A white-flower variety of sweet william was used in these tests, which took place over the period of about 1½ years. In no case did the controls, maintained in numbers equal to the inoculated plants, become infected. The total number of potted plants inoculated during this time was 52, all of which developed the disease. In addition to these, larger numbers of seedlings were successfully inoculated.

In each of two inoculation series, 12 pink and red varieties of carnation, *Dianthus caryophyllus* L., were included in the tests. None of these developed symptoms, although they were retained nearly 2 months after the sweet william plants had died.

A vascular fusarium wilt of carnation, caused by *F. oxysporum* f. *dianthi* (Prill. et Del.) Sny. et Han., has been known for about 40 years, but no report of a similar disease of sweet william, another common species of the same genus, has appeared in the literature. Furthermore, inoculation experiments have indicated that the pathogen of the newly found wilt of sweet william does not attack carnation. In view of these facts, coupled with knowledge of the high degree of host specificity typically shown by the vascular fusaria, it is concluded that the sweet william fungus is a form distinct from *F. oxysporum* f. *dianthi.* Consequently, in keeping with a procedure of *Fusarium* classification presented recently,[2] it is proposed that the form of *Fusarium oxysporum,* which causes the vascular wilt of sweet william, *Dianthus barbatus,* be designated as *F. oxysporum* f. *barbati,* n. f.—W. C. SNYDER, Division of Plant Pathology, University of California, Berkeley, California.

Willow Blight in British Columbia.[1]—Willow blight has been known in the eastern United States and Canada for more than a decade. Clinton[2] first observed the disease in Connecticut in 1927 and Harrison[3] began his studies of it in Nova Scotia during the same year. However, what was undoubtedly the same disease began to attract attention in Prince Edward Island as early as 1925,[4] although the cause was not determined. Since then willow blight has destroyed large numbers of willows in the Maritime Provinces and in Quebec on the north shore of the Bay of Chaleur and along the St. Lawrence River. In the United States it is destructive in New England and has been reported from Maine, New Hampshire, Vermont, and North Carolina in 1937.[5] The present acccount appears to be the first report of its occurrence on the Pacific Coast.

In the spring of 1940 a series of twig cankers were collected by G. E. W. Clarke from a willow growing in the hospital grounds at Abbotsford, B. C. The cankers were covered with the acervuli of the conidial stage of *Physalospora miyabeana* Fuk. and on two of them perithecia of this pathogen were also found. No leaves were received but on one small twig *Fusicladium saliciperdum* (All. and Tub.) Tub. was fruiting freely.

When Abbotsford was visited on September 18 the tree was found to be heavily infected, and several smaller trees, which had originated from cuttings taken from it and planted on an adjoining property, were also affected. The tree in the hospital grounds was about 20 years old, and had grown from a cutting taken from a willow on a private property about one mile from the hospital. The parent tree had been removed and, as the owner had died several years previously, it was not possible to secure any information concerning its origin. The disease was first observed about 3 or 4 years ago. Since a thorough search in Abbotsford and vicinity failed to disclose the presence of other diseased willows it was decided that the four infected trees should be destroyed and, through the co-operation of the owners, this was accomplished. More extensive scouting in the lower Fraser River valley and about Victoria on Vancouver Island did not reveal any further instances of willow blight.

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[1] Contribution No. 668 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Clinton, G. P. A new disease of willows appears in Connecticut. U. S. Dept. Agr. Pl. Dis. Rptr. 11: 87–88. 1927.

[3] Harrison, K. A. Willow blight. Canada Dept. Agr. Rept. Dom. Bot. (1928): 34–36. 1929.

[4] McCurry, J. B., and A. J. Hicks. Forest and shade tree diseases. Canada Dept. Agr. Ann. Rept. Prev. Pl. Dis. Dom. Canada 5: 68. 1925.

[5] Wood, J. I., and N. W. Nance. Diseases of trees. U. S. Dept. Agr. Pl. Dis. Rptr. Supp. 110: 251–253. 1939.

Material collected in September showed cankers 2 to 4 cm. in length on twigs 2 to 7 mm. in diameter. The cankers had girdled the smaller twigs and the death of the terminal shoot and its leaves had followed. The surface of the cankers was thickly studded with the acervuli of *Physalospora miyabeana* but no *Fusicladium* was present then (Fig.1). The constant association of two organisms in willow blight is a remarkable fact. The symptoms caused by the two fungi are identical and there has been disagreement as to which is the primary parasite. In England Nattrass[6] regards *Fusicladium saliciperdum* as secondary to *Physalospora miyabeana;* Dennis[7] finds that *F. saliciperdum* is not the cause of any disease in willow; while

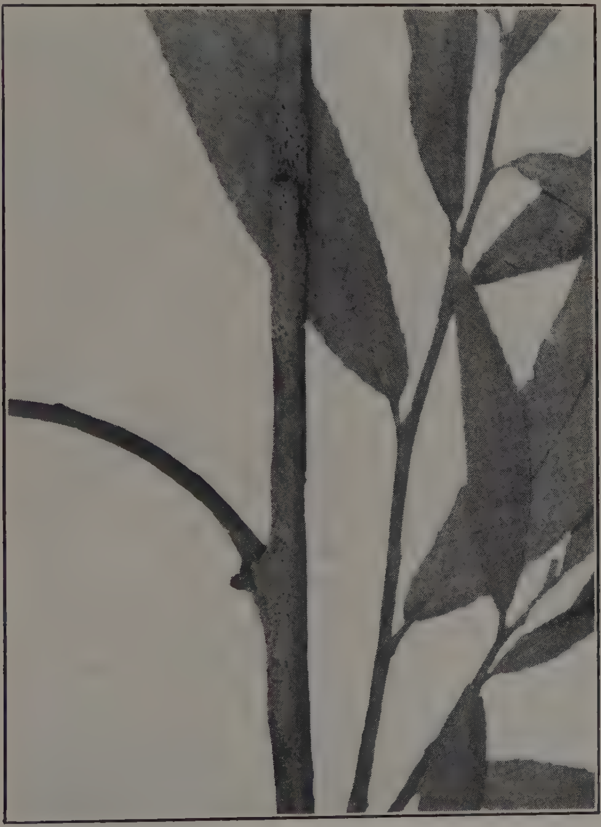

FIG. 1. Willow branch showing two cankers caused by *Physalospora miyabeana.* Fruiting bodies appear as black dots on the light gray surface of the cankers. The twig at the left has been killed. ×1.5.

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[6] Nattrass, R. M. The Physalospora disease of the basket willow. Trans. Brit. Mycol. Soc. 13: 286–304. 1928.

[7] Dennis, R. W. G. The black canker of willows. Trans. Brit. Mycol. Soc. 16: 76–84. 1931.

Brooks and Walker[8] conclude that both fungi are capable of causing serious injury. In the United States Clinton and McCormick[9] believe that *F. saliciperdum* is the principal cause of willow blight with *P. miyabeana* causing less serious injury later in the season. In Canada, Harrison[10] finds that both fungi can cause serious injury, but that *F. saliciperdum* initiates the blight a little earlier in the spring than *P. miyabeana*. In the present instance it was largely the *Physalospora* which was present on the specimens examined.—I. L. CONNERS, A. W. MCCALLUM, and J. E. BIER, Ottawa, Ont., and Victoria, B. C.

A Low-temperature Basidiomycete Causing Early Spring Killing of Grasses and Legumes in Alberta.[1]—A low-temperature basidiomycete, not yet identified, has been found to cause extensive killing of certain grasses and alfalfa in Alberta about the time of the first spring thaw. This fungus was first isolated in 1931 from turf grasses severely damaged by snow-mould.[2] Later, the junior writer obtained the same fungus from a destructive crown rot of alfalfa and tested its pathogenicity on various legumes.

Insofar as the early spring killing of grass is concerned, this basidiomycete may be classed as a snow-mould. The affected patches of turf are definitely smaller than those caused by *Fusarium* spp. Also, on these affected areas are found light gray hyphae instead of a pinkish mycelium. In a field test, plants of Kharkov winter wheat, from seed planted early in September, 1940, and inoculated with the basidiomycete in November, were destroyed during the early part of March, 1941 (Fig. 1, A). Also, 22 species of grasses were killed in the same test. *Bromus inermis* Leyss was the only one that appeared to be partially resistant. The pathogen was isolated from diseased tissue much more readily at 5° than at 20° C.

During early spring this basidiomycete also kills alfalfa. Dark-brown, crown lesions, which sometimes extend down the root, are produced. Usually the plants are killed in irregular patches, and these areas enlarge from year to year. Damage ranging from a trace to the destruction of 50 per cent of the plants has been found in many alfalfa fields in central and northern Alberta. The pathogen can be isolated fairly readily from diseased crown tissues incubated at a temperature near freezing. Inoculation experiments (Fig. 1, B) have shown it to be the most virulent fungus yet isolated from crown and root tissues of alfalfa in Alberta. All varieties so far tested of alfalfa, sweet clover, red clover, alsike, and white dutch clover, have been completely killed in the early spring.

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[8] Brooks, F. T., and M. M. Walker. Observations on *Fusicladium saliciperdum*. New Phytol. 34: 64–67. 1935.

[9] Clinton, G. P., and F. R. McCormick. The willow scab fungus. Conn. Agr. Exp. Stat. Bull. 302: 443–469. 1929.

[10] Harrison, K. A. Willow disease investigations—Maritime Provinces. Canada Dept. Agr. Rept. Dom. Bot. (1930): 37–38. 1931.

[1] Contribution No. 669, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa.

[2] Broadfoot, W. C. Experiments on the chemical control of snow-mould of turf in Alberta. Sci. Agr. 16: 615–618. 1936.

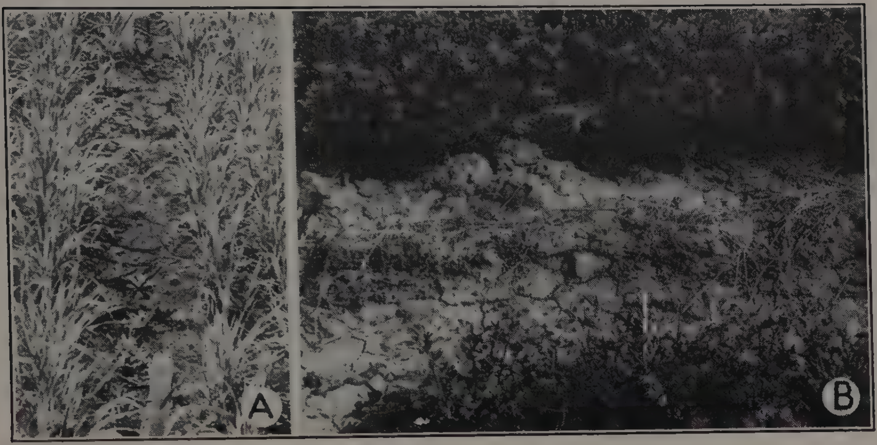

FIG. 1. Early spring killing of (A) Kharkov winter wheat, and (B) Grimm alfalfa, by the low-temperature basidiomycete. Note healthy plants surrounding killed areas. Inoculum was applied the previous fall.

This pathogen grows readily on most nutrient media at temperatures from 0 to 18° C., producing a fluffy white, sterile mycelium. Cardinal temperatures for growth are about −4, 15, and 26 degrees C. Sclerotia, fruiting bodies, and spores have not been found on the diseased host plants, and many attempts to produce them in culture under varying conditions of temperature, light, moisture, and nutrient media have failed. The characteristic clamp connections of the mycelium and the occasional production of small rudimentary sporophores on culture media indicate that this fungus is a basidiomycete. It may be related to *Typhula*, but the complete absence of sclerotia excludes it from those species studied by Remsberg.[3, 4]—W. C. BROADFOOT and M. W. CORMACK, Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton.

Virus Antagonism, Natural Host Resistance, and the Acquired-immunity Concept with Reference to Plants.—In recent years, discussions on "acquired immunity" in plants have centered around the virus diseases, especially the mosaics and ring spots. Price,[1] in a paper dealing with this subject, comments on the writer's[2] first record of virus antagonism in plants as follows: "It appears unlikely that McKinney regarded the protection by the light-green-mosaic virus as acquired immunity, for he merely mentioned the experiment casually and did not report any further study of the matter."

Contrary to Price's statement, reference to the literature will show that

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[3] Remsberg, Ruth E. The snow mould of grains and grasses caused by *Typhula itoana* and *Typhula idahoensis*. Phytopath. **30**: 178–180. 1940.

[4] Remsberg, Ruth E. Studies in the genus *Typhula*. Mycologia **32**: 52–96. 1940.

[1] Price, W. C. Acquired immunity from plant virus diseases. Quar. Rev. Biol. **15**: 338–361. 1940.

[2] McKinney, H. H. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. Jour. Agr. Res. [U.S.] **39**: 557–578, 1929.

the writer did make further study of the matter.[3, 4, 5, 6] Furthermore, at the time of the first report, enough information was already in hand to make the writer reluctant to interpret the reaction as acquired immunity. In a later report,[4] the subject of possible acquired immunity in tobacco was discussed, but the idea that we are dealing with *bona fide* acquired immunity in the case of Nicotiana virus 1 and its yellow mutant Type A, was not accepted wholeheartedly for the reasons that seem to be rather clearly stated in the conclusions to that paper.

Among the vertebrates there are many outstanding examples of both recovery and acquired immunity from virus and other infectious diseases. Infected tissues eventually become healthy, virus-free and resistant to reinfection for a long period or throughout life. In plants, however, infected tissues seldom, if ever, actually recover and become free of an infective agent.

In the mosaics, the so-called acquired immunity may be regarded as a type of virus antagonism or selective antibiosis, more or less comparable to the aversion reaction that Hoppe[7] described in corn plants and in culture plates, when certain pairs of *Diplodia zeae* strains were introduced.

Studies on tobacco ring spot have borne out Valleau's[8] conclusion, that plants do not outgrow the disease. Under the usual conditions of plant culture, the disease passes from the acute phase (ring spots, lesions, and oak-leaf patterns) to a less evident chronic phase in the subsequent virus-infected leaves. Furthermore, infected plants have given low yields of seed.[8]

In these studies, the diffuse chlorosis associated with low temperature, as described by Valleau,[8] and also a mottled and a necrotic-spot form of this reaction, appeared only in growing leaves in certain varieties, and when the infected plants were provided with ample amounts of fertile soil. It was found that the continuation of the typical acute symptoms is favored when succulent growing leaves are wiped with large quantities of virus when the plants have entered the period of maximum growth rate. In certain selections of early maturing tobacco, as high as 70 per cent of the plants expressed acute symptoms in all of the leaves that appeared after the onset of the disease. Usually, but one to four leaves expressed acute symptoms when young seedlings were inoculated before the period of rapid growth, and usually very weak or no local or systemic symptoms appeared when virus

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[3] McKinney, H. H. The inhibiting influence of a virus on one of its mutants. Science 82: 463–464. 1935.

[4] McKinney, H. H. Evidence of virus mutation in the common mosaic of tobacco. Jour. Agr. Res. [U.S.] 51: 951–981. 1935.

[5] McKinney, H. H. The "acquired immunity" test and its limitations for establishing relationship between virus mutants, and nonrelationship between distinct viruses. Third Int. Cong. Microbiol. (1939): 316–317. (New York.) 1940.

[6] McKinney, H. H. Virus genes. Proceedings 7th Int. Genet. Cong. (1939) 200–203. (University Press. Cambridge.) 1941.

[7] Hoppe, Paul E. Intraspecific and interspecific aversion to *Diplodia*. Jour. Agr. Res. [U.S.] 53: 671–680. 1936.

[8] Valleau, W. D. Experimental production of symptoms in so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopath. 31: 522–533. 1941.

was wiped on slow growing leaves having the characteristic thick, leathery texture.

It appears that tobacco varieties, for the most part, do not possess a very high degree of susceptibility to ring spot, and that a complete understanding of the disease depends in large measure, on an understanding of the several factors that influence the expression of natural host resistance.

In recent years the term acquired immunity has been applied to so many diverse types of reactions that it is losing its significance and there is little, if anything, that can be said in favor of some of the acquired-immunity types that have been proposed. Price[1] cites three types of active acquired immunity: (1) chronic-disease type, (2) carrier type, and (3) sterile type. The writer proposes that type 3 be recognized as *bona fide* active acquired immunity and that types 1 and 2 be discarded, at least by phytopathologists. In the case of type 1, this proposal is based on the fact that application of the term to the domination of one mosaic virus over another serves no real purpose when we take into account the fact that the so-called protecting virus continues to multiply throughout the life of the plant and induces a disease that in some instances is far more devastating to the plant than the disease against which it is presumed to protect.[6] In the case of type 3, the proposal is based on the view that the term immunity should be reserved for those cases in which a given virus or other infectious agent cannot multiply in a given organism. So-called symptomless carriers and mild reactors, then, represent degrees of resistance, or tolerance, and susceptibility, because it seems highly improbable that carriers are totally free from symptoms. It seems far more reasonable to believe that plants that support a virus, bacterium, a fungus, or protozoan will manifest at least microscopic and biochemical signs of disorder.—H. H. MCKINNEY, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

A ROOT DISEASE OF JEFFREY AND PONDEROSA PINE REPRODUCTION[1]

ALVER J. OLSON[2,3]

(Accepted for publication April 5, 1941)

In May, 1934, a disease causing the death of Jeffrey pines (*Pinus jeffreyi* Mur.) was found at Lasco, on the eastern slope of the Sierra Nevada Mountain range in northern California, by K. A. Salman, Bureau of Entomology and Plant Quarantine, of the United States Department of Agriculture. The roots of the dying trees were involved, and, concluding that the disease was not caused by insects, Salman brought it to the attention of the San Francisco office of the Division of Forest Pathology, Bureau of Plant Industry. Ernest Wright of that Division, together with Salman, commenced in May, 1934, a series of investigations that continued until late summer of that year.

Cultures were made from affected roots of fading trees, and Wright consistently isolated one certain fungus. In June, 1934, the bases of 3 Jeffrey pines on the Stanislaus National Forest, California, were inoculated with cultures of the fungus isolated at Lasco. No infection resulted. Wright realized that inoculations should also be made into roots, but was obliged to give up the problem on leaving the State at that time.

The disease was brought to the attention of the writer in the spring of 1937 by Willis W. Wagener, Senior Pathologist with the San Francisco office of the Division of Forest Pathology. Observations and experiments were initiated by the writer in May, 1937, and continued until June, 1940.

The disease has thus far been found only at Lasco. The pine regions of California have been extensively inspected by Wagener and Salman who state that they have not seen the disease elsewhere.

Affected trees are found in localized centers that are thinly scattered over an area of approximately 7,500 acres, lying just west of Lasco. The areas on which the disease is found were logged off under U. S. Forest Service supervision during the years from 1919 to 1930, inclusive. The elevation is approximately 5,500 feet above sea level. The ground is fairly level, with a few knolls and slopes, and many rocks of volcanic origin are present.

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[1] A revised dissertation submitted to the Graduate School of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Timber sales supervisor, The Red River Lumber Company, Westwood, California.

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The ground is quite moist during the spring of the year, because of late-melting snows; this condition usually continues until the latter part of July, after which the soil gradually dries out. With the exception of a few small spots along streams, the area is well drained.

The conifers growing on this area are: ponderosa pine (*Pinus ponderosa* Dougl.); Jeffrey pine (*P. jeffreyi*); white fir (*Abies concolor* Lindl. and Gord.); some lodgepole pine (*P. contorta* var. *murrayana* Engelm.); and a few sugar pines (*P. lambertiana* Dougl.) in 2 small, localized areas.

DESCRIPTION OF THE DISEASE

The disease has been found to affect the roots of Jeffrey and ponderosa pines, but, to date, has not been observed on any other conifers.

The youngest affected tree observed by the writer was 5 years old, the oldest was 17. In 1934 Salman and Wright found 3 diseased Jeffrey pines that were much older, and measured from 14 to 16 in. in diameter at breast height. The roots of these trees manifested typical disease symptoms and Wright isolated the fungus that eventually proved to be the pathogen.

Localized centers of diseased trees always are observed in the immediate vicinity of the old stumps on the cutover area. Affected trees have been found so close to the neighboring stump that they actually touched it; others have been found at various distances from the stump, the most distant being 19 ft.

Fifty-four stumps in the immediate vicinities of diseased trees were examined. All were either Jeffrey pine or ponderosa pine. The bark of the stumps was examined to differentiate between the two, and a confirmation was given by noting the presence or absence of the typical aroma of fresh-cut wood of the former. Sixteen of the stumps were ponderosa pine and 38 were Jeffrey pine.

Affected trees show no external symptoms during the early stages of the disease. During the later stages the affected tree begins to fade; the normal green color of the needles fading to a paler green, then to a whitish-green. The needles then quickly turn yellow as the diseased tree dies (Fig. 1, A). The needles later become brown and still later fall, leaving the skeletons of the dead trees standing clustered around the old stumps (Fig. 1, C and D). An exudate of yellowish-white pitch, which may flow onto the surrounding soil or duff, is found on the root collars of dead and dying trees.

The first symptoms (paling of affected trees) are first observed from early May to late June, depending on the weather. They appear in summer and normally prevail up until late September, when frost first generally occurs at Lasco and the disease becomes less active. Warm weather, however, persisted late into the fall of 1939, and the initial symptoms were observed as late as early December.

An exudate of yellowish-white pitch is found on the surfaces of diseased roots. The steles of affected roots are heavily infiltrated with pitch (Fig. 2, D and G). Freshly-killed roots can be easily sliced with a knife, but after they have been dead for some time the steles become hard and glassy from hardening of the infiltrated pitch. Steles of smaller roots become translucent because of heavy pitch infiltrations. The extreme point of advance of a lesion is commonly in the center of the stele.

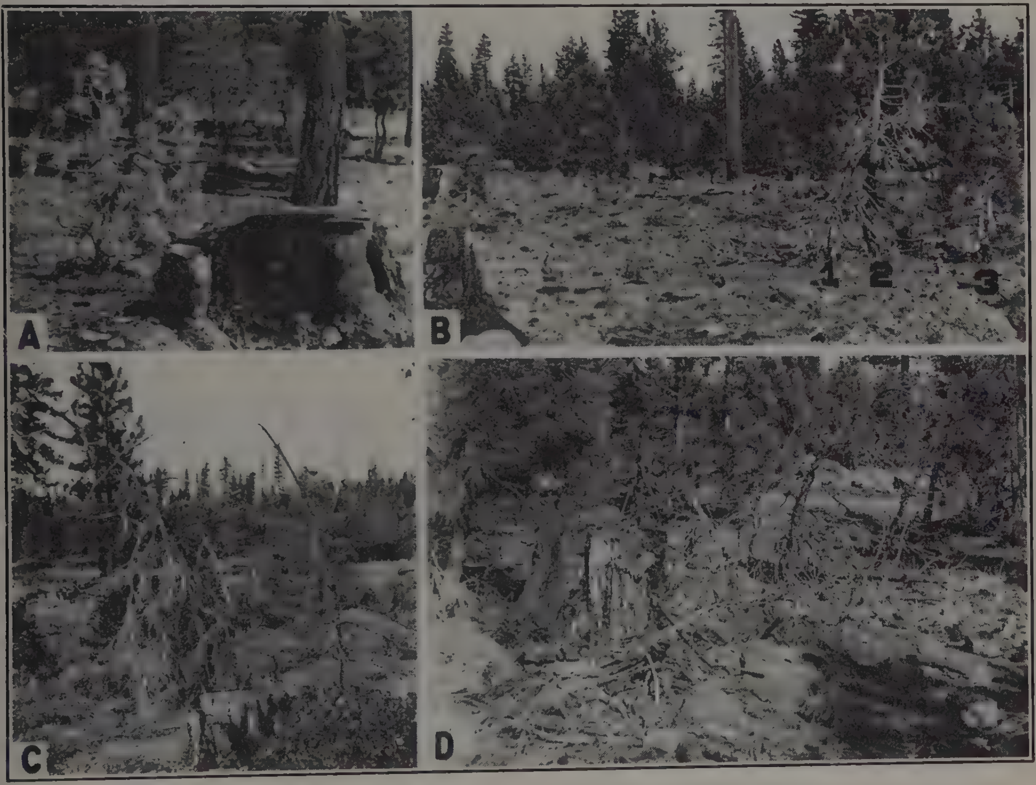

FIG. 1. A. Jeffrey pine sapling dying of the root disease in the immediate vicinity of a Jeffrey pine stump. B. Infection center in immediate vicinity of a Jeffrey pine stump: 1, a Jeffrey pine killed by the root disease; 2, a ponderosa pine dying of the root disease, the needles on the lower branches are yellow; 3, a seemingly healthy Jeffrey pine, but one of its roots is affected with the root disease. C. Two Jeffrey pines killed by the root disease, in the immediate vicinity of a ponderosa pine stump. D. Young Jeffrey and ponderosa pines, in the immediate vicinity of an old Jeffrey pine stump, killed by the root disease.

Near the advancing margin of the lesion, the pitch infiltration sometimes is found more abundant in summer than in spring wood, accentuating its grain (Fig. 2, G2).

The root systems of 20 affected trees were completely exposed by excavating the earth from around them. Some of the trees were dead or dying; others showed no external symptoms of the disease, but their roots were found to be affected. In every case one, or more, of the roots of the affected tree was in close contact with either an old, dead root of the neighboring stump (Fig. 2, B), or with a diseased root of another affected pine (Fig. 2, F). In some cases both the infected and infecting root were in simple contact (Fig. 2, B), while in a few cases the former had grown around the latter in a half-clasping manner. Twelve of the 20 root systems were in contact

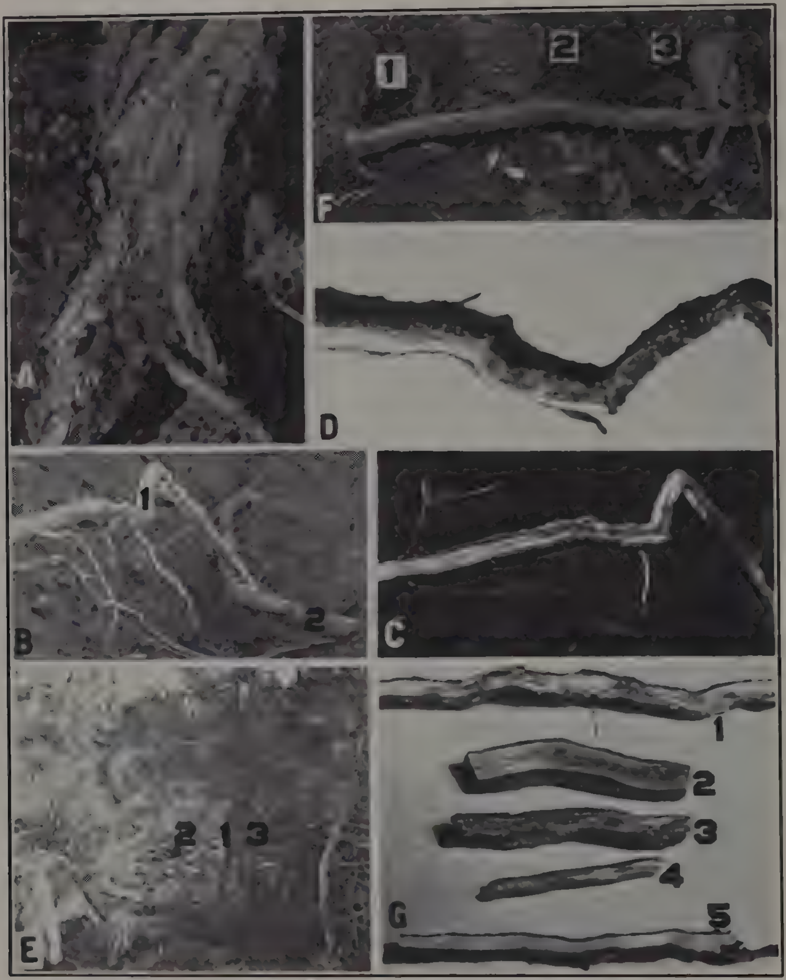

FIG. 2. A. Root collar of dying ponderosa pine of figure 1, B; wood was becoming pitch-infiltrated and exudate of whitish pitch was showing at lower left. B. Jeffrey pine root; 1, in contact with dead root of ponderosa pine stump, 2. C, D. Same Jeffrey pine root as in B, cut open to show pitch infiltration. E. Saplings in vicinity of a Jeffrey pine stump: 1, seemingly healthy ponderosa pine; 2, ponderosa pine dying of the root disease; 3, seemingly healthy Jeffrey pine. F. Same saplings as in E: roots of 2, pitch infiltrated; lateral root of 1 infected, pitch infiltration extending nearly its entire length; the two small clasping roots of 3 had become infected and pitch-infiltrated. G. Jeffrey pine roots: 1, showing pitch infiltration resulting from inoculation with *Cunninghamella* 6 months earlier at point indicated with arrow; 2, 3, 4, roots found infected in the forest—heavy infiltration of pitch in summer wood of 2 accentuates grain of wood; 3 and 4, hard and flinty because of infiltrated pitch; 4, a translucent stele; 5, a normal healthy Jeffrey pine root.

with a root, or roots, of the stump; 5 were in contact with diseased roots of affected pine trees, and 3 with roots of both the stump and diseased trees.

In 6 observed cases only 1 root of the diseased tree was affected. In 4 of these cases this affected root was in close contact with a root of the neighboring stump (Fig. 2, B), while in 2 cases the affected root was in close contact with an affected root of another diseased tree (Fig. 2, F). In 2 of the cases where only 1 root was affected the lesions were quite young, being about 2 in. long. These small lesions were found on the infected root at the point where it came in contact with the infecting root.

Although only one root may be affected at first, the entire root systems were found involved in cases where the tree had died. The pitch-infiltrated condition extended throughout the entire root systems and up into the root collars (Fig. 2, A). In young trees up to 17 years of age, the pitch infiltration extended from about 1 to 4 in. above ground level; but, verbally, Wagener stated that it may extend a foot or so above ground level on diseased trees that are about 16 in. in diameter.

Diseased trees may be found on one or more sides of a stump, or they may completely surround it. The trees nearest the stump usually become affected first; those farther away in the same radial direction later become successively affected (Fig. 1, B); this is not, however, an invariable rule.

The heavy infiltration of pitch is apparently a physiological reaction, and, in some cases, the marked infiltration seems to have overcome the pathogen. In such instances the affected roots were hard and flinty. Other roots in the same root systems were unaffected, and these maintained the affected trees in apparently normal health. No fungus growth was obtainable from the flinty, pitch-infiltrated parts, although microscopic examination showed within them some mycelium.

ECONOMIC IMPORTANCE OF THE DISEASE

The disease affects Jeffrey and ponderosa pines, both of which are valuable as timber trees. To date the number of trees killed has been small. Most of these were saplings.

The majority of trees on the Lasco area are young; in spots, this growth is quite dense. When the disease becomes established in such areas (Figs. 1, D, 2, E), where there is considerable interlocking of root systems (Fig. 2, F), there is greater chance for infection and reinfection, so that the disease might be able to wipe out all the Jeffrey and ponderosa pines on some areas. Should this happen, these areas would then probably become covered with other kinds of plants not subject to the disease.

As the trees on the area become older and the root systems more extensive, other contacts will be made between the root systems of neighboring trees, so that there will be more opportunities for infection and reinfection.

ISOLATION AND INOCULATION EXPERIMENTS

Material from diseased roots of affected Jeffrey and ponderosa pines was plated out on potato-dextrose agar. Some of these roots were from trees that had begun to fade; others, though infected, were from trees that showed no aboveground symptoms. In most cases the root material was surface-sterilized by immersing for 5 to 8 min. in 1–1,000 $HgCl_2$ solution before plating. In some cases the root material was plated out after removing it aseptically from or near the advancing margins of the pitch infiltrations.

At first, *Cunninghamella* sp. and *Penicillium* spp. were commonly isolated, but these fungi were given little attention, as it was assumed that they were secondary saprophytic invaders. *Pythium* sp. was isolated from the cortical regions of 3 roots of a dying Jeffrey pine. Since several species of *Pythium* are known parasites, this *Pythium* sp. from the Jeffrey pine roots was tested for pathogenicity.

Jeffrey pine seedlings, 4 months old, were first used to test the *Pythium* sp. F. S. Baker had grown them from seed at low elevation (Berkeley, California) and out of the natural range of the species the seedlings had been transferred to earthenware pots.

Twenty of the seedlings were inoculated on Feb. 22, 1938, with a pure culture of *Pythium* sp. In making the inoculations, the cortex was scraped from the tap root of each seedling on an area approximately $\frac{1}{8}$ in. square, exposing the stele. Several grains of wheat on which was growing a pure culture of *Pythium* sp. were fastened with adhesive tape to the root at the scraped spot. Five seedlings were treated with sterilized wheat grains as checks. Within 30 days 8 of the inoculated and 2 of the check seedlings had died. In all cases there was necrosis of root tissue but no infiltration of pitch. Plate cultures of the diseased roots showed *Fusarium* spp. and *Cylindrocarpon* sp., but in no case was *Pythium* sp. recovered.

Inoculations were next made on Jeffrey pine in the forest and under natural conditions. The Red River Lumber Company granted the writer use of an isolated tract of cutover timber land on its holdings at Nubieber, California, approximately 4,600 feet above sea level, and approximately 50 miles due north of Lasco. The experimental trees were of the natural reproduction on the area.

Ten roots on 10 healthy Jeffrey pine trees, 4–7 ft. high and 1–3 in. in diameter, were inoculated with a pure culture of *Pythium* sp. on May 29, 1938. To make the inoculations, a portion of a root was bared; a hole about $\frac{1}{4}$ in. in diameter and $\frac{1}{4}$ in. deep was cut into the root with a pocket-knife. Several *Pythium*-infested grains of wheat were stuffed into the hole, and then covered with waterproof adhesive tape. Five additional roots were treated with sterilized wheat grains as controls. All treated roots were examined on October 2, 1938. No evidence of killing or pitch infiltration could be found, the holes made for inoculation purposes being nearly covered over with a new growth of healthy tissue. Further experiments with *Pythium* sp. were not attempted.

Isolation work from diseased root material from the Lasco area was intensified during the summer and fall of 1938: material being collected from widely-separated parts of the area. Material was collected from trees that had begun to fade and from those whose roots were found affected, but whose aboveground parts showed no symptoms of the disease. *Cunninghamella* sp. was the only fungus consistently isolated from the affected roots, and it was recovered from over 100 trees from all parts of the area. The fungus was isolated from the cambial regions and from the steles, at the advancing margins of pitch infiltrations of affected roots. When young lesions were plated out it was obtained in pure culture.

Recalling Wright's consistent isolation of one certain fungus from diseased root material, a culture of the *Cunninghamella* was referred to him. He pronounced the fungus identical with the one he had isolated in 1934.

On August 7, 1938, the soil in 10 pot cultures of Jeffrey pine seedlings was inoculated by stirring into it a wheat-grain culture of *Cunninghamella* sp. Three check pot cultures of Jeffrey pine were inoculated with sterilized wheat grains. The seedlings were maintained at Berkeley during the course of the experiment. The plants were examined on December 18, 1938, and again on February 5, 1939, but no indications of disease could be found. The seedlings used were part of the lot received from Professor Baker and were 11 months old at the time of inoculation. In all subsequent inoculations the seedlings used were from this same lot.

Fourteen seedlings were taken to Nubieber from Berkeley. On September 21, 1938, 10 of these were inoculated with a pure culture of *Cunninghamella* sp. In making these inoculations, the cortex was removed from the tap-root of each seedling on an area approximately $\frac{1}{8}$ in. square. Several *Cunninghamella*-infested wheat grains were placed in the earth alongside these scraped spots. The 4 remaining seedlings were similarly treated with sterilized wheat grains as checks. The plants were examined on October 16, 1938, and again on November 3, 1938, but no indications of disease could be found. On January 2, 1939, it was observed that 6 of the 10 inoculated plants were fading. Lesions were found on the roots of all 6 plants; nearly all of the root system of each seedling being involved. The cortical regions were soft and dead, and the steles were turning brown, but there was no infiltration of pitch. The roots of the 4 remaining inoculated plants and 4 checks were examined. Smaller lesions were found on the roots of all plants, including the checks. All of the roots were surface-sterilized and plate cultures were made from them. *Fusarium* spp., *Cylindrocarpon* sp., *Trichoderma* sp. and *Macrosporium* sp. were isolated from both the inoculated roots and from the checks, but no *Cunninghamella* sp. was recovered.

In February and March, 1939, 50 Jeffrey pine seedlings, $1\frac{1}{2}$ years old, were inoculated with pure cultures of *Cunninghamella* sp. The tangential-slit method of Meinecke (5) was used in making these and all subsequent inoculations, unless otherwise stated. The earth was carefully removed from around the root selected for inoculation, the smallest particles being brushed away with a camel-hair brush. A slit about $\frac{1}{8}$ in. long was made through the cortex. By carefully manipulating a curved-point scalpel through this slit, a small pocket was made at one side of the slit between the cortex and the stele. A small fragment of a wheat grain, on which was growing a pure culture of *Cunninghamella* sp., was inserted into this pocket, which was then closed by pressing together the sides of the slit. In some of these inoculations the root was then wrapped with waterproof adhesive tape at the point of inoculation, but this wrapping was dispensed with in later inoculations. Fifteen seedlings were treated with sterilized wheat grains as checks.

Ten inoculated seedlings were removed from their pots and examined 6 weeks after inoculation. Nine showed no symptoms, while the 10th showed a streak of yellowish pitch in the center of the stele, extending about an inch above and below the point of inoculation. Three check seedlings examined at the same time showed no symptoms. The 10 inoculated roots and the 3 check roots were all surface-sterilized and plated out. In all platings of inoculated material, a piece of root about ⅝ in. long, in the immediate vicinity of the inoculation slit, was discarded without plate culturing. No fungi were isolated from the checks or from inoculated roots showing no symptoms. The 10th inoculated root, which bore a pitchy infiltration, yielded a pure culture of *Cunninghamella* sp. up to a distance of about 1¼ in. both above and below the point of inoculation.

Ten more inoculated seedlings and the 3 corresponding checks were examined 2 months after inoculation. Again the root of 1 inoculated seedling was pitch-infiltrated, whereas the roots of the remaining 9 inoculated seedlings and the 3 check roots showed no infiltration. A pure culture of *Cunninghamella* sp. was recovered from the infiltrated root up to distances of about 1¾ in. both above and below the point of inoculation, whereas no fungi were isolated from the remaining 9 inoculated roots or from the 3 check roots.

The remaining 30 inoculated seedlings and the 9 checks were allowed to remain untouched, at Berkeley, until February 19, 1940. By this time none of the seedlings had died, and all were to all appearances healthy. On February 19, 1940, the roots of 10 inoculated seedlings and of their 3 checks were examined. Two of the inoculated seedlings had old pitch streaks about 2 in. long in the centers of their tap roots, the streaks extending about an inch above and below the points of inoculation. The rest of the inoculated roots and the check roots had no such pitch streaks. All of the roots were surface-sterilized and plated *Fusarium* spp. and *Verticillium* sp. were isolated from the pitch-streaked roots. No fungi were recovered from the other 8 inoculated roots or from the check roots.

On February 9, 1940, 10 seedlings were inoculated at Berkeley with *Cunninghamella* sp. and 2 were treated with sterilized wheat grains as checks. These seedlings were now 2½ yr. old. On March 3, 1940, 8 of the inoculated seedlings and the 2 check seedlings were examined. The inoculated tap roots of the 8 inoculated were infiltrated with a light-yellow pitch to a distance of about ¾ in. both above and below the points of inoculation. The 2 check roots showed no pitch infiltration. All of the roots were surface-sterilized and plated out. *Cunninghamella* sp. was recovered from all 8 inoculated, pitch-streaked roots to a distance of about 1¼ in. both above and below the points of inoculation. No fungi were isolated from either of the check roots. The production of the typical symptoms of pitch infiltration, and the recovery of *Cunninghamella* sp. from the inoculated roots establishes this fungus as the organism causing the root disease. The two remaining inoculated roots were examined on May 25, 1940.

TABLE 1.—*Roots of Jeffrey pine seedlings inoculated at Berkeley, California, with Cunninghamella sp.*

Seedlings' age at time of inoculation	Individuals inoculated	Checks	Date inoculated	Date examined	Instances typical symptoms produced	Instances *Cunninghamella* sp. reisolated
Yr.	*Number*	*Number*				
1	10[a]	3	8/ 7/38	2/ 5/39	0	.....
1	10[b]	4	9/21/38	1/ 2/39	0	0
1½	10	3	2/ 4/39	3/18/39	1	1
1½	10	3	2/ 6/39	4/ 6/39	1	1
1½	10	3	2/ 8/39	2/19/40	2	0
1½	10	3	2/14/39	Roots not examined, seedlings examined superficially.		
1½	10	3	3/ 4/39	Roots not examined, seedlings examined superficially.		
2½	10	3	2/ 9/40	8 on 3/ 3/40 2 on 5/25/40	8 2	8 0

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[a] Soil surrounding roots, and not roots themselves, inoculated with *Cunninghamella* sp.
[b] Seedlings taken to Nubieber, California, for this experiment.

The central portions of the steles, equivalent to the entire steles as of February 9, 1940, before the seedlings had commenced spring growth, were heavily infiltrated with pitch. The cambium of both seedlings had been killed next to the inoculation to distances of about ½ in. both above and below, and ⅛ in. on either side of the points of inoculation. The remaining healthy portions of cambium had remained normally active, so that thick layers of spring wood had been laid down since February 9, covering the pitch-infiltrated portions of the steles completely, with the exception of narrow creases on the inoculated sides, where the cambium had been killed. The new spring wood showed no signs of pitch infiltrations, and the seedlings were apparently healthy. Microscopic examination revealed mycelium in the pitch-infiltrated portions of the steles, but there was no mycelium in the new, spring tissue. Plate cultures were made from the roots. No fungi were isolated from either root, suggesting that the invading organism had been overcome by the pitch.

Field inoculations by the tangential-slit method or by contact were made with *Cunninghamella* sp. on the experimental plot at Nubieber.

Twenty roots on 20 separate Jeffrey pines were inoculated with a pure culture of *Cunninghamella* sp. by the tangential-slit method on May 8, 1939.

The trees ranged from 3 to 20 ft. in height and from 1 to 9 in. in diameter. Four roots on 4 similar Jeffrey pines were treated with sterilized wheat grains as checks.

Ten of the inoculated roots and 2 check roots, examined on Sept. 9, 1939, showed an infiltration of pitch in each inoculated root to distances varying from 1 to 4½ in. both above and below the points of inoculation (Fig. 3, A–B). There were no pitch infiltrations in either of the check roots. All roots were surface-sterilized and plate-cultured, the material plated from the inoculated roots being taken from the advancing margins of the pitch infiltrations. *Cunninghamella* sp. was recovered from all 10 inoculated roots, the recoveries being made from material up to 4½ in. from the points of inoculation. No fungi were isolated from either of the check roots.

The remaining 10 inoculated roots and the 2 check roots were examined on November 4, 1939. All 10 inoculated roots showed the typical pitch-infiltrated symptoms, while the 2 check roots showed no symptoms. The 2 check roots and 8 of the 10 inoculated were all surface-sterilized and plate-cultured, the 2 remaining inoculated roots being used for dissection. *Cunninghamella* sp. was recovered from all 8 inoculated roots from tissue up to 5½ inches both above and below the points of inoculation. No fungi were isolated from the check roots. The results obtained in this experiment again prove the pathogenicity of *Cunninghamella* sp.

Roots of 6 ponderosa and 6 sugar pines on the Nubieber plot were inoculated with *Cunninghamella* sp. on May 14, 1939. Roots of 2 trees of each species, subjected to sterilized wheat grains, served as controls. The trees used were from 3–7 ft. tall and from 1–4 in. in diameter, for both species. All roots were examined on September 24, 1939. The checks of both species and the 6 inoculated sugar-pine roots showed no symptoms, but the 6 inoculated ponderosa pine roots showed the typical pitch infiltrations, extending both above and below the points of inoculation to distances of about 3 in. *Cunninghamella* sp. was recovered from the advancing margins of the pitch infiltrations in all 6 of these latter cases, again proving the pathogenicity of the fungus.

On May 21, 1939, *Cunninghamella* sp. was inoculated into the bases of 6 Jeffrey pines at points about 1 in. above ground level, the trees being from 3 to 8 ft. tall and from 1–4 in. in diameter. The tangential-slit method of inoculation was again employed, the wrapping with adhesive tape being dispensed with. Three similar trees were treated with sterilized wheat grains as controls. All trees were examined on November 5, 1939, but no symptoms were observed either on the inoculated bases or on the checks.

The roots of 6 white firs and 6 incense cedars (*Libocedrus decurrens* Torr.) were inoculated with *Cunninghamella* sp. on May 21, 1939. Roots on 2 trees of each species were treated with sterilized wheat grains as checks. The trees treated ranged from 3 to 6 ft. in height and from 1–3 in. in diameter for both species. All roots were examined on November 11, 1939, but there were no infiltrations of pitch or evidence of lesions on either the inoculated roots or on the checks.

Some experiments were undertaken to test the ability of the fungus to cause infection through contact. Sections of healthy roots from 4–6 in. long and from $\frac{1}{4}$–$\frac{3}{4}$ in. in diameter were placed in 500-cc. Erlenmeyer flasks. One hundred cc. of water was added to each. The flasks were then plugged and autoclaved 1 hr. at 20 lb. pressure. Flasks containing root sections of Jeffrey, ponderosa, and sugar pine, incense cedar, and white fir were thus prepared. Pure cultures of *Cunninghamella* sp. were then introduced into

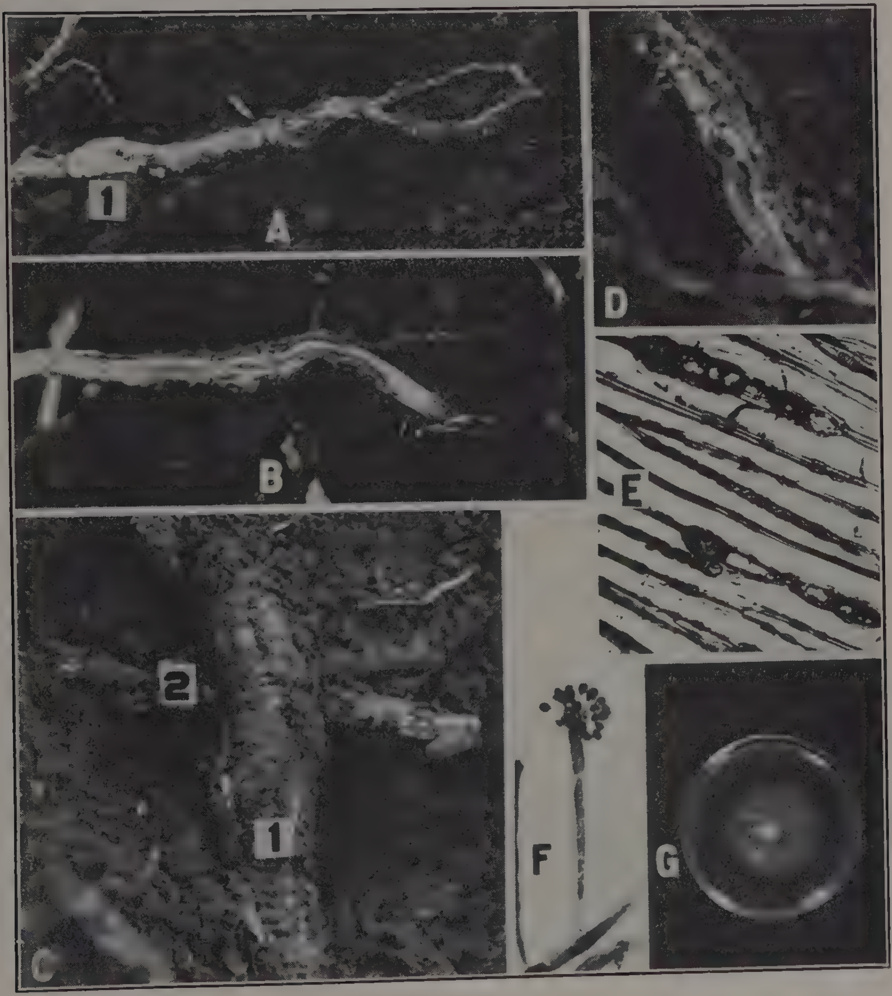

FIG. 3. Jeffrey pine root inoculated at point 1 with a pure culture of the *Cunninghamella.* B. Same root as in A cut open 4 months after inoculation and showing typical symptoms of pitch infiltration. C. Two sections of ponderosa pine roots: 2, inoculated with the *Cunninghamella* and placed in contact with a healthy, uninjured Jeffrey pine root 1. D. Same Jeffrey pine root as in C, cut open 5½ months after inoculation and showing typical symptoms of pitch infiltration. E. Longitudinal tangential section from advancing margin of pitch infiltration in Jeffrey pine root inoculated with the *Cunninghamella,* showing mycelium of the fungus in tracheids and ray cells. × approx. 81. F. Conidiophore of *Cunninghamella meineckella.* × approx. 162. G. Seven-day-old culture of *Cunninghamella meineckella* on potato-dextrose agar.

these flasks. Additional flasks containing sterilized, noninoculated root sections were prepared as checks. About a month later there was abundant mycelium on the inoculated root sections; and these were then used to make contact inoculations. It should be borne in mind that the growth of *Cunninghamella* sp. on all of these root sections was strictly saprophytic.

The contact inoculations were made on the Nubieber plot on May 28, 1939. The trees—Jeffrey pines—varied from 4 to 10 ft. high and from $1\frac{1}{2}$ to 4 in. in diameter. The roots inoculated varied from $\frac{5}{8}$ to $1\frac{1}{2}$ in. in diameter. To make a contact inoculation, either one or two *Cunninghamella*-inoculated root sections were placed in close contact with, but were not fastened to, a healthy, uninjured root (Fig. 3, C). For a check, a sterilized, noninoculated root section was placed in close contact with a healthy root. Two Jeffrey roots were contact-inoculated with *Cunninghamella*-inoculated root sections of ponderosa pine, and a check was made with another Jeffrey root and a noninoculated ponderosa root section. Two more Jeffrey pine roots were contact-inoculated with *Cunninghamella*-inoculated root sections of Jeffrey pine, and a check was set up with another Jeffrey root and a noninoculated Jeffrey root section. Two more Jeffrey pine roots were contact-inoculated with *Cunninghamella*-inoculated root sections of sugar pine, and a check was set up with another Jeffrey pine root and a noninoculated sugar pine root section. Two more Jeffrey roots were contact-inoculated with *Cunninghamella*-inoculated root sections of white fir, and a check was set up with another Jeffrey root and a noninoculated white fir root section. Two more Jeffrey pine roots were contact-inoculated with *Cunninghamella*-inoculated root sections of incense cedar, and a check was set up with another Jeffrey pine root and a noninoculated incense cedar root section.

All treated roots were examined on November 5, 1939. There were pitch infiltrations in all 10 Jeffrey pine roots that had been contact-inoculated with *Cunninghamella*-inoculated root sections. This infiltration of pitch ex-

TABLE 2.—*Roots of coniferous saplings inoculated in the forest at Nubieber, California, with Cunninghamella sp.*

Conifer inoculated (slit method unless noted)	No. individuals inoculated	No. checks	Date inoculated	Date examined	Instances typical symptoms produced	Instances *Cunninghamella* sp. reisolated
Jeffrey pine	20	4	5/ 8/39	10 on 9/ 9/39 10 on 11/ 4/39	10 10	10 8[a]
Ponderosa pine	6	2	5/14/39	9/24/39	6	6
Sugar pine	6	2	5/14/39	9/24/39	0	......
Jeffrey pine[b]	6	3	5/21/39	11/ 5/39	0	......
White fir	6	2	5/21/39	11/11/39	0	......
Incense cedar	6	2	5/21/39	11/11/39	0	......
Jeffrey pine[c]	10	5	5/28/39	11/ 5/39	10	10

[a] Only 8 of the 10 roots plated out.
[b] Inoculations made into bases of saplings.
[c] Root-contact inoculations.

tended from ½ to 2 in. both above and below the points on the Jeffrey pine roots where they had been in contact with the *Cunninghamella*-inoculated root sections (Fig. 3, D). *Cunninghamella* sp. was reisolated from the advancing margins of the pitch infiltrations as much as 2 inches from the points of contact. No pitch infiltrations were found in any of the 5 Jeffrey pine check roots. This experiment proves that the disease can be spread by root contacts.

MYCELIUM IN DISEASED ROOT TISSUE

Root material from the advancing margins of the pitch infiltrations was first surface-sterilized in preparing it for microscopic examination. Each piece was then split longitudinally through the pitch infiltration, one half being plated out and the other half being fixed in alcohol-formalin-acetic acid fixative No. 1, as outlined by Rawlins (7). If the portion plated yielded a pure culture of the *Cunninghamella,* its corresponding half was sectioned. Sections were made free-hand or with a sliding microtome without imbedding. The sections were treated with xylol to remove the pitch, which would otherwise mask the mycelium.

The sections were examined after staining by Cartwright's method (7). The hyphae of the *Cunninghamella* are commonly found in cambium cells, ray cells, and tracheids, where they occur in the cell lumina. They are branched, of variable diameter, and coenocytic when young, becoming sparingly septate with age. At first hyaline, they become brownish later. They commonly pass through the bordered pits (Fig. 3, E), but penetrate the cell wall directly when passing from one living cell to another.

THE CAUSAL ORGANISM

The pathogen has been identified by the writer as a species of *Cunninghamella.*

The described species of the genus *Cunninghamella* have been found living saprophytically on a variety of substrata. Several species have been isolated from soil samples (1, 3, 6); others have appeared in laboratories as agar contaminants (1). Matruchot (4) found *C. africana* living saprophytically on dried camel dung in the French Sudan. Saccardo (8) observed *C. albida* on dead roots of lemon trees. Some species have been found growing saprophytically on dead botanical specimens, especially on material from the tropics (2, 9). A review of literature on the genus *Cunninghamella* uncovers no previous report of pathogenicity in this genus.

The species of *Cunninghamella* causing the root disease of Jeffrey and ponderosa pines is a minute form, being much smaller than any described, with the exception of *C. microspora* (Rivolta) Matruchot (1). It has not been possible to obtain a culture of *C. microspora,* this species seeming to be out of culture, so that no direct comparison of the two fungi can be made. The available description (1) of *C. microspora* is meager and affords only a few points for comparison. The size of the conidiophore heads and the width of the spores are approximately the same for both fungi. The spore walls of the pine-root *Cunninghamella* are smooth, whereas those of *C. microspora* are finely papillate. The conidiophore heads of the pine-root *Cunninghamella* are sterigmatate; those of *C. microspora* are minutely papillate. No parasitism is reported for *C. microspora.* Since it is not possible to identify the pine-root *Cunninghamella* as one of the described species, the name **Cunninghamella meineckella** sp. nov., is proposed, in honor of E. P. Meinecke, pioneer forest pathologist of California. The description of the fungus follows.

Cunninghamella meineckella sp. nov.

Mycelium patens, caespem subtilem formans, hypharum ramis intertextis, eburneum, in aetate fuscum; hyphae aseptatae, in aetate parce septatae, diametro variabiles, praecipue 2.1–5.7 μ, medio circa 3.6 μ; conidiophora erecta, simplicia, aseptata, in aetate septata, longitudine variabilia, 57.5–402.5 μ, medio circa 250 μ, diametro 4.8–7.9 μ, medio circa 6.0 μ; conidiophorum capitula rotunda, diametro 9.3–21.2 μ, medio circa 16.5 μ, 2.5 sterigmata 2.3–4.6 μ longa, praecipue 25 μ, unisporiata; sporidia hyalina, parietibus laevibus, obovata, basaliter subapiculata, 4.5–7.2 μ × 5.3–11.1 μ, medio circa 5.6–8.0 μ.

Hab. in radicibus atque in regionibus ad caudices transitionalibus Pini jeffreyi Mur. et Pini ponderosae Dougl.

For the convenience of mycologists, the type specimen has been deposited with the University of California Herbarium, Berkeley, California.

Cunninghamella meineckella is moderately slow-growing; when diseased root material is plated out, from 5 to 10 days frequently elapse before the mycelium can be seen growing out of the root material.

A test was made to determine the growth-temperature relationship for this fungus. *Cunninghamella meineckella* was planted on the centers of potato-dextrose agar plates, and incubated at 0.1 to 36.8 degrees Centigrade at approximately 3-degree intervals. The temperature was checked at 8-hour intervals. The cultures were run in triplicate and the diameters of the fungus colonies were measured at the end of 9 days. The results are as follows:

Temperature in °C.	0.1	3.0	6.3	10.0	13.0	16.0	18.7
Average diameters of colonies in cm.	0.0	0.0	0.37	0.95	2.0	2.15	5.75
Temperature in °C.	22.0	25.5	28.2	30.8	33.2	36.8	
Average diameters of colonies in cm.	8.2	9.0	4.35	1.1	0.3	0.0	

At the end of two weeks, colonies averaging 0.3 cm. in diameter were found on the plates held at 3.0°, but no growth was found on the plates held at 0.1° nor on those held at 36.8°. At the end of 4 weeks, colonies averaging 0.2 cm. in diameter were found on the plates held at 0.1°, but no growth was found on the plates held at 36.8°.

SUMMARY

A disease of Jeffrey and ponderosa pines on a cutover forest area at Lasco, Lassen County, California, induces an infiltration of pitch in diseased roots and root collars, and may ultimately result in the death of affected trees.

The disease, to date, has been of little economic importance.

Diseased trees always are found in the immediate vicinity of old stumps. Affected root systems are in contact either with roots of the stumps or with diseased roots of other affected trees.

A species of *Cunninghamella* was consistently isolated from diseased roots.

Inoculations of this species of *Cunninghamella* into roots of Jeffrey pine seedlings up to 1 yr. of age produced no infection that could be attributed to this fungus. Inoculations into roots of seedlings 1½ years old produced the typical symptoms in 2 cases out of 20 inoculations, and the fungus was reisolated from the diseased roots. Inoculations into roots of seedlings 2½ years old produced the disease in 100 per cent of the roots inoculated.

Inoculations of this species of *Cunninghamella* into roots of Jeffrey and ponderosa pine saplings in the woods produced the disease in 100 per cent of the roots inoculated. Inoculations of the fungus into roots of sugar pine, white fir, and incense cedar saplings failed to produce the disease in any of the inoculated roots.

Contact inoculations in the woods on Jeffrey pine saplings produced the disease on 100 per cent of the roots inoculated, proving that the disease can be spread by root contacts.

The fungus causing the root disease is described as **Cunninghamella meineckella** sp. nov.

The optimum growth temperature for this fungus ranges from 22.0 to 25.5° C. Slow growth takes place at the minimum of 0.1°, and there is some growth at 33.2°, but none at 36.8° C.

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LITERATURE CITED

1. ALCORN, GORDON D., and CHARLES C. YEAGER. A monograph of the genus *Cunninghamella* with additional descriptions of several common species. Mycologia 30: 653–658. 1938.
2. BLAKESLEE, A. F. Two conidia-bearing fungi. Bot. Gaz. 40: 161–170. 1905.
3. LENDNER, A. Les Mucorinées de la Suisse. Beitr. zur Kryptflra. der Schweiz 3: 158–161. 1911.
4. MATRUCHOT, L. Une Mucorinée purement conidienne, *Cunninghamella africana.* Ann. Myc. 1: 45–60. 1903.
5. MEINECKE, E. P. Facultative heteroecism in *Peridermium cerebrum* and *Peridermium Harknessii.* Phytopath. 10: 286. 1920.
6. PAINE, F. S. Studies on the fungus flora of virgin soils. Mycologia 19: 251–265. 1927.
7. RAWLINS, T. E. Phytopathological and botanical research methods. John Wiley and Sons, Inc. (New York.) 1933.
8. SACCARDO, P. A. Michelia 2: 288. 1882.
9. STADEL, O. Über einen neuen Pilz, *Cunninghamella Bertholletiae.* Rev. Mycol. Centralbl. 1: 218–219. 1912.

THE PROBABILITY LAW IN COTTON SEEDLING DISEASE

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INTRODUCTION

The study of seedling disease or damping-off has engaged the attention of numerous investigators particularly during the past decade. It is patent that these studies have been fruitful, especially as regards the practical accomplishment of damping-off control by seed treatments. Attention also has been given to certain theoretical aspects of the damping-off problem. The findings include the following principles:

1. The treatment of seed with suitable disinfestant chemicals under most conditions results in a decrease in seedling disease and consequent increase in the percentage of seedling survival.
2. Such chemicals may operate by destruction or inhibition of pathogens on the seed surface (exclusively so in the case of formaldehyde, for example), or by destruction or inhibition of pathogens in the soil adjacent to the seed, or by a combination of the two processes.
3. The treatment of seed with corrosive agents, such as concentrated sulphuric acid, may result in a decrease in seedling disease by shortening the period of susceptibility, alone or in addition to seed-surface disinfestation (2).
4. The benefits of seed treatments are most pronounced at medium levels of infection potential, and progressively decrease as the infection potential approaches extremely high or extremely low levels (3).
5. In general, the dust treatments are more effective against seed-borne pathogens, than against strictly soil-borne seedling pathogens, such as *Rhizoctonia* (4).

In virtually all of the seedling-disease studies, the investigators have recorded the total percentages of seedling survival or the yields of unit areas, and used these values in determining the practical effects of seed treatments. In the present study of damping-off in cotton, attention has been focussed on the law of probability as it relates to the survival of seedlings at various infection potentials. With this approach it has been possible to arrive at certain interpretations of the behavior of seedlings and the functions of disinfestants that are not apparent when the more customary methods of analysis are used. The results have been previously summarized (1).

MATERIALS AND METHODS

This study is largely a by-product of the 1939 and 1940 uniform cotton seed-treatment tests carried on at the Oklahoma Agricultural Experiment Station farm at Perkins, Oklahoma, in cooperation with the other southern experiment stations, under the auspices of the Cotton Disease Council. In addition to the nontreated, delinted, and Ceresan-treated seed provided for the uniform tests, seed of Oklahoma provenience was used and the treatments were extended to include acid delinting, followed by flotation-grading, and, in 1940, by subsequent Ceresan treatment. Five plantings were made at various dates, each planting consisting of 20 seed types × 4 replications of 50-foot rows × 2 planting rates (5 seeds per hill and 2 seeds per hill, with 10″ spacing between hills). The seedling counts were made about 10 days after emergence, and, in addition to recording the total number of surviving seedlings per row, a record was kept of the number of surviving seedlings in each hill. The latter data were required for a study of the probability of seedling emergence. In addition, a few greenhouse experiments were performed using the same types of seed in a partially controlled environment.

CALCULATION OF THE PROBABILITY OF EMERGENCE

The point of departure of this study was the question: Does each diseased seedling behave as a discrete unit, with no injurious effect on adjacent seedlings, or does the presence of a diseased seedling in a hill operate to endanger and destroy adjacent, otherwise normal, seedlings? If each diseased seedling is an independent unit there should be a normal or random distribution of seedling failures among the hills. If, on the other hand, the diseased seedling is a hazard to adjacent seedlings, the random distribution would no longer obtain, but there would be a skewness in the distribution curve, resulting from an excessively large proportion of hills with more seedling failures than are consistent with a normal distribution.

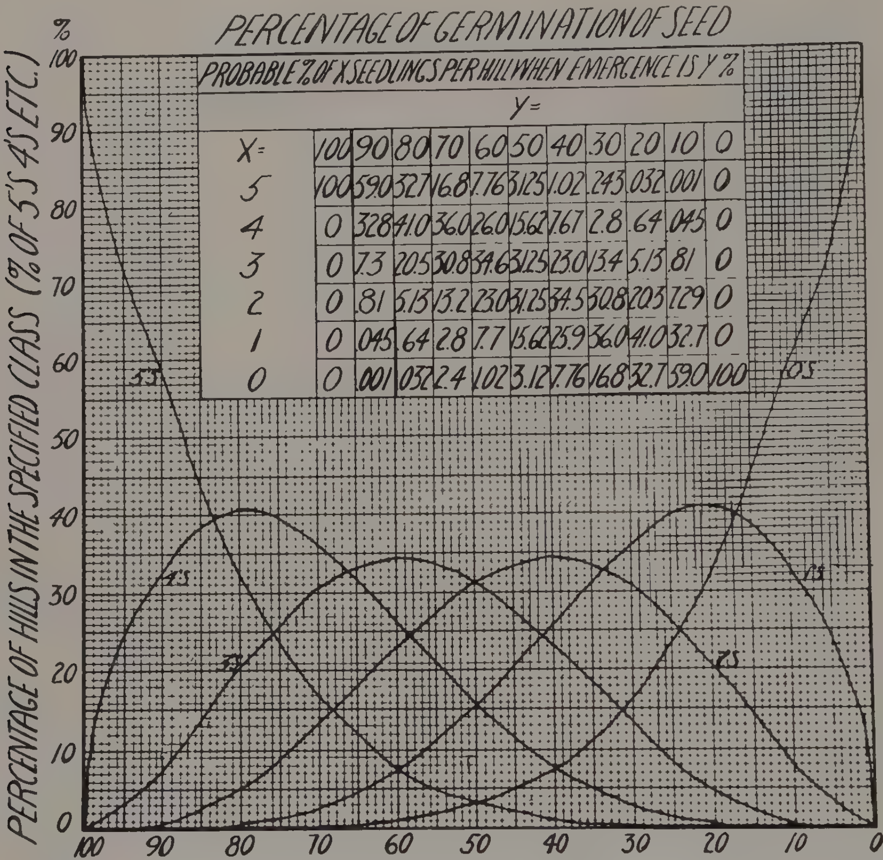
If 30 per cent of the seedlings in a given block of 100 hills proved defective, it was assumed that, with a normal distribution, there would be as many hills with 5, 4, 3, 2, 1, or 0 defective seedlings as there would be boxes with 5, 4, 3, 2, 1, or 0 black balls if a thoroughly mixed collection of 500 balls, 70 per cent of which are white and 30 per cent black are distributed at random in 100 boxes, 5 balls to the box.

The mathematical formula applying to this case is obtained by expansion of the binomial equation as follows: If the number of hills = x, seeds are planted at the rate of 5 per hill, the resulting successful emergence is y per cent (y/100), and the number of seedlings emerging in any given hill = z, then:

If z =:	*The number of hills with z seedlings will be:*
5	$1 \cdot (y/100)^5 \cdot x$
4	$5 \cdot (y/100)^4 \cdot (100-y/100)^1 \cdot x$
3	$10 \cdot (y/100)^3 \cdot (100-y/100)^2 \cdot x$
2	$10 \cdot (y/100)^2 \cdot (100-y/100)^3 \cdot x$
1	$5 \cdot (y/100)^1 \cdot (100-y/100)^4 \cdot x$
0	$1 \cdot (100-y/100)^5 \cdot x$

To simplify calculation, the curves for the values y = 0, 10, 20, . . . 100 were plotted as in figure 1, and intermediate values of y could then be measured on the curves.

Using values thus obtained it becomes possible to plot curves showing the probable frequency of hills yielding 5, 4, 3, 2, 1, or 0 healthy seedlings when the gross emergence percentage is any given value. This yields the family of curves illustrated in figure 2. The bell-shape, normal frequency curve applies when emergence is 50 per cent, and with departures from that value in either direction the curve is correspondingly skew.



PROBABLE % OF X SEEDLINGS PER HILL WHEN EMERGENCE IS Y %

	Y=										
X=	100	90	80	70	60	50	40	30	20	10	0
5	100	59.0	32.7	16.8	7.76	3.125	1.02	.243	.032	.001	0
4	0	32.8	41.0	36.0	26.0	15.62	7.67	2.8	.64	.045	0
3	0	7.3	20.5	30.8	34.6	31.25	23.0	13.4	5.13	.81	0
2	0	.81	5.13	13.2	23.0	31.25	34.5	30.8	20.5	7.29	0
1	0	.045	.64	2.8	7.7	15.62	25.9	36.0	41.0	32.7	0
0	0	.001	.032	2.4	1.02	3.12	7.76	16.8	32.7	59.0	100

FIG. 1. Master curves for determining the calculated probable percentage of hills containing given numbers of seedlings, when seeds are planted 5 per hill and display any given emergence percentage.

If we now consider a given planting and observe, for example, that 80 per cent of the planted seeds have successfully emerged, according to the curve in figure 2, approximately 36 per cent of the hills should show 5 normal seedlings, 40 per cent should have 4, 20 per cent should have 3, 4 per cent should have 2, and none of the hills should have 1 or 0 healthy seedlings. Turning then to the emergence counts it is possible to plot on the same axes the percentage of hills of each type actually obtained. The two curves will either coincide or fail to do so. This procedure was followed in the case of each seed type in each planting. The following section deals with the results obtained and their interpretation.

EXPERIMENTAL RESULTS AND INTERPRETATION

The second planting of 1939 was followed by weather conditions admirably adapted to cotton seedling development, all plantings yielding good stands with 50–78 per cent of the seeds producing healthy plants. Figure 3 presents the results of emergence, expressed as percentages of hills with 5, 4, 3, 2, 1, or 0 seedlings (solid line) in comparison with the expected random distribution of hills of each class (dotted line).

The curves illustrate that in this instance a regular and close agreement was obtained between the observed and the calculated values. All of the seed lots were infested with cotton-seedling pathogens, and half of them were treated with Ceresan, which kills or inhibits such organisms on the seed surface. In no case is there an important deviation between calculated and observed values. There is no such deviation if treated seed are compared with nontreated, or if any one seed lot is compared with any other.

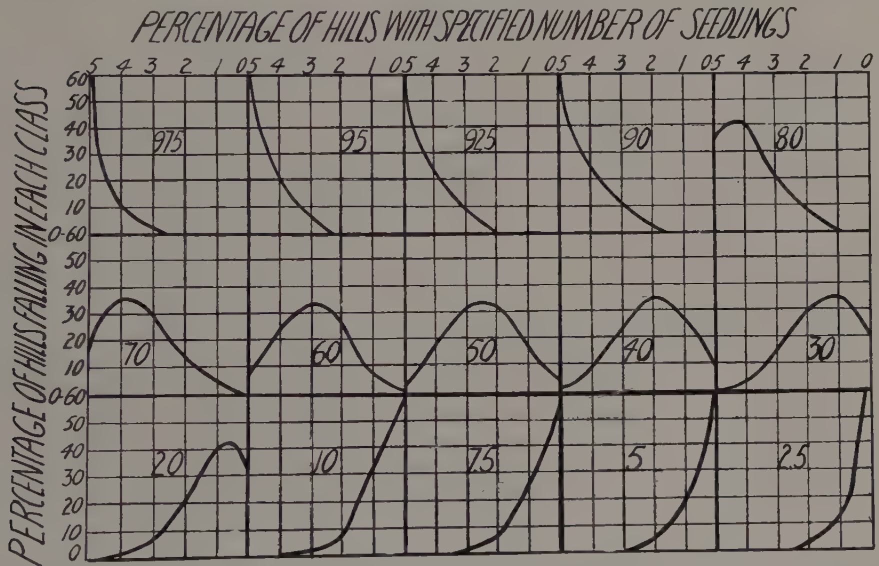


FIG. 2. The calculated probable percentage of hills containing 5, 4, 3, 2, 1, or 0 seedlings when the seeds are planted 5 per hill and display certain given percentages of emergence.

These data permit two conclusions: (a) the agreement of the curves shows that the probability distributions derived from expansion of the binomial equation are applicable to analysis of seedling emergences in the field; (b) that the distribution of seedling failures is a random distribution indicates that, in this experiment, diseased seedlings are not proving a hazard to nearby healthy seedlings, since such an effect would produce a higher proportion of hills with 1 and 0 seedlings and a correspondingly lower proportion of hills with 2 and 3 seedlings.

The third planting of 1939 was followed by cool, moist conditions, less favorable for cotton-seedling development. The field emergences varied between 14 per cent and 76 per cent (Fig. 4).

It is obvious that in this case there is no such close agreement between the calculated and observed emergence distributions as occurred in the second planting. Moreover, the observed values all depart from the calculated ones in the same manner, *i.e.*, there is a greater proportion of hills with 5 healthy seedlings and of those with no seedlings, and a corresponding smaller proportion having intermediate numbers of seedlings than is consistent with a normal distribution of seedling failures. The same seed lots were used in both plantings, including lots that were heavily infested with such virulent pathogens as the anthracnose organism, and lots that were presumably free of surface infestation after Ceresan treatment and that showed high laboratory germination percentages.

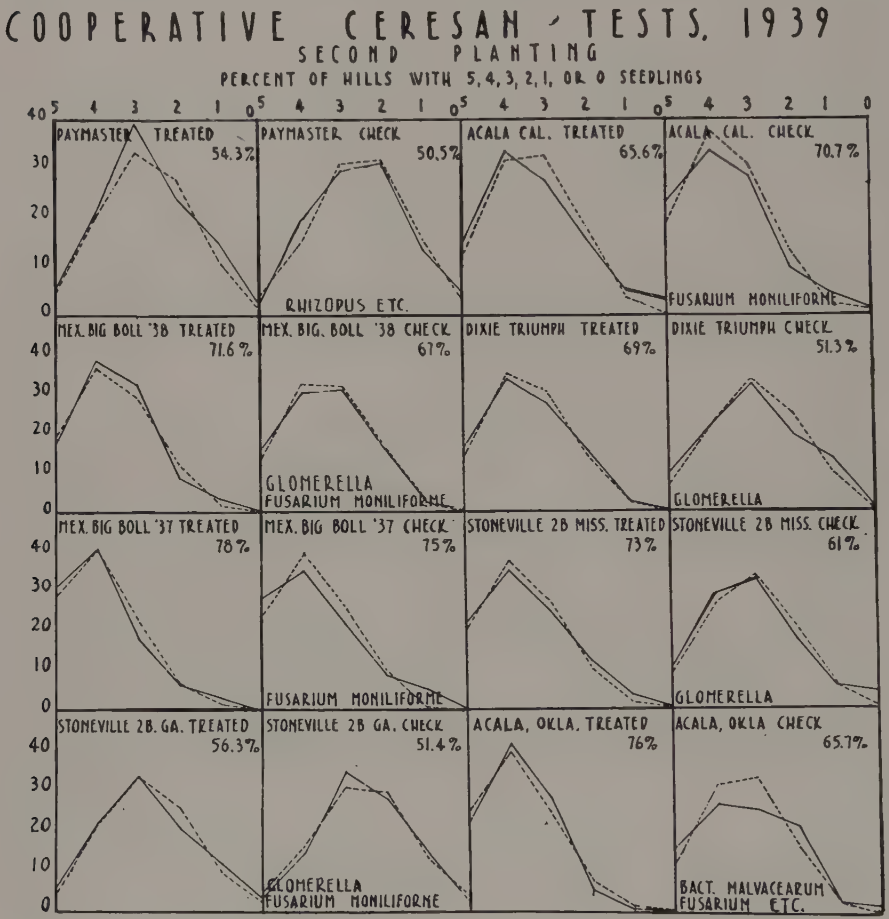


FIG. 3. Percentages of hills with 5, 4, 3, 2, 1, or 0 seedlings in cotton planting under relatively low disease potential. Dotted lines give values obtained by calculation; solid lines gives values obtained from field counts.

A study of figure 4 shows that the deviation from normal distribution is not peculiar to any type of seed. The type of deviation indicated occurs uniformly in treated and nontreated seed, and in heavily and lightly infested seed. This planting also included a lot of Oklahoma seed that had been acid-delinted and graded by flotation. This seed lot ("Oklahoma heavy" in Fig. 4) was essentially free of any external or internal infestation. Laboratory germination tests of this lot had repeatedly yielded 99 per cent or 100 per cent healthy germination. Yet, this disease-free seed lot showed the deviation from the normal distribution to the same extent and in the same manner as the other types of seed. If the results of this

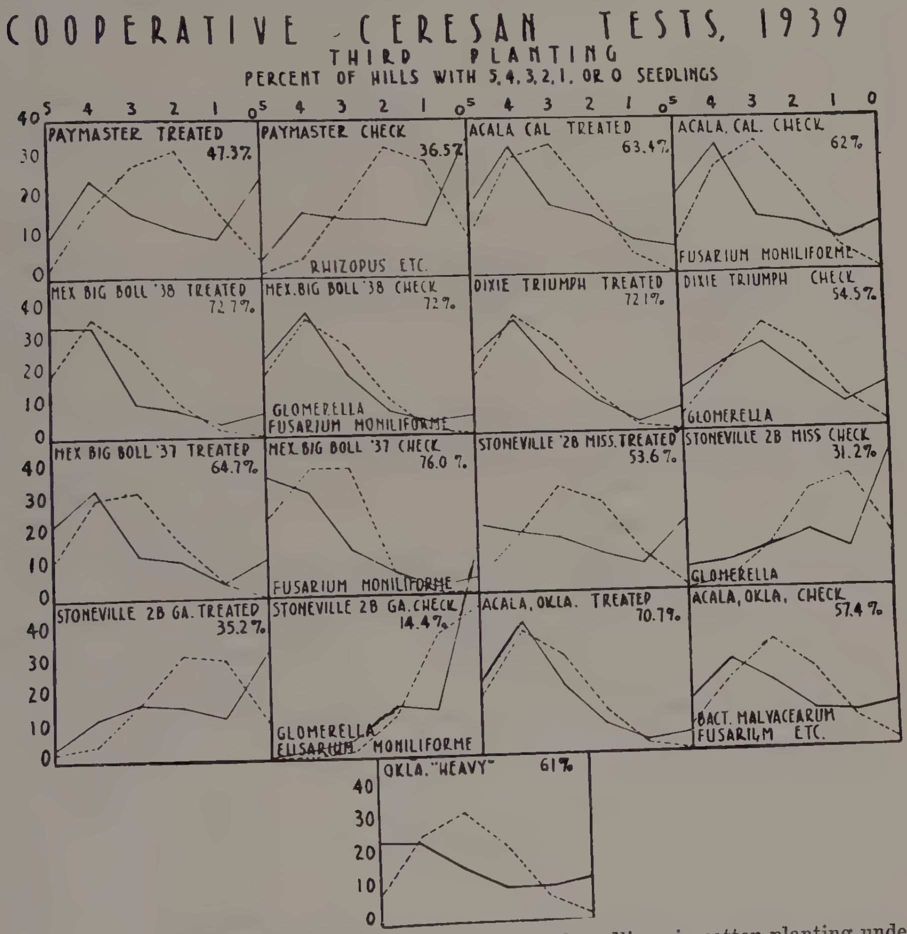


FIG. 4. Percentages of hills with 5, 4, 3, 2, 1, or 0 seedlings in cotton planting under conditions favorable for rhizoctonia infection. Dotted lines give values obtained by calculation; solid lines give values obtained from field counts.

experiment are expressed pathologically: (a) regardless of the presence or absence of seed infestation, if as many as 2 seedlings in a hill were failures, such failure extended to include one or more of the potentially healthy seedlings in that hill; and (b) this hazard occurred in all seed types under one set of environmental conditions, and was absent from all seed types in another environment. There are various possible explanations of this behavior, most of which can be excluded by the available evidence.

It might be assumed that the abnormally high percentage of hills with no surviving seedlings is due to the "all or nothing" character of field accidents. Entire hills, as such, are wiped out by careless cultivation, by the misstep of a mule, or by the feeding of rodents on sprouting seed. But such accidents would affect hills of all classes equally, and not be largely restricted to the hills with 2 and 3 successful seedlings, failing to affect those with 5 seedlings, as is true in the present instance. Moreover, such field accidents would not normally affect all plantings in some experiments and none in others. Field accidents may thus be eliminated as the principal explanation of the deviations in distribution.

There is the remaining possibility that these deviations are due to the development of seedling disease in a non-normal distribution, *i.e.,* in such a way that the loss of 2 or 3 seedlings in a hill predisposes that hill to further loss. Such seedling disease might arise from external or internal seed infestations or from a soil-borne infestation. Either type of seed infestation may be eliminated as cause for the deviation because it is observed to equal extent in nontreated and treated seed, in noninfested, lightly infested, and heavily infested seed, in delinted, as well as fuzzy, seed, in both the internally infested floating fraction, and the disease-free sinking fraction of delinted seed, and in seed with good germination as well as that which germinates poorly.

There remains the possibility that the preponderance of total failures in hills originally containing 2 or 3 diseased seedlings is due to soil-borne seedling disease. Were this the case, the deviation from normal should be uniformly evident on all types of seed under environmental conditions favoring soil-borne infection, and uniformly lacking from all seed types under a more favorable environment. This is the observed fact. The deviations as seen in figure 4 occurred equally in all seed types in the third planting of 1939 and in both plantings of 1940, while they were absent from all seed types in the second planting of 1939, and occurred to only slight extent in all seed types of the first planting of that year.

This interpretation is borne out by the results of isolations from diseased seedlings of these plantings, which yielded the exclusively soil-borne pathogen *Rhizoctonia solani* Kühn as the predominating organism associated with the seedling disease. That rhizoctonia attacks are of an "all or nothing" nature is a matter of common observation, the disease regularly occurring in spots of varying dimensions, in which all plants in the spot are destroyed. In the case of *Rhizoctonia* the presence of a diseased seedling, marking the advance of the fungus in the soil, is clearly a hazard to adjacent seedlings. Attack by *Rhizoctonia* would, then, produce the deviation in the distribution of diseased seedlings of the type observed in figure 4, with an excessive proportion of hills that are total failures.

From the preceding analysis it was indicated that: (a), under favorable field conditions with a minimum of rhizoctonia attack, seedlings from infested seed did not prove hazardous to adjacent seedlings from healthy seed; and, (b), under field conditions marked by rhizoctonia activity, there was an excessive number of seedling failures in the poorer hills, related to the rhizoctonia attack, but unrelated to seed infestation. Further experiments were undertaken in order to obtain additional evidence on these questions.

In an attempt to observe directly the effect of a diseased seedling on an adjacent healthy one, pots of sterile soil were planted, each with a ''healthy'' and a ''diseased'' seed, with suitable controls; and this was followed by observation of the percentage of healthy emergence of the ''healthy'' seed in presence and absence of ''diseased'' seed. The ''healthy'' seed was acid-delinted and graded by flotation, to give a fraction that regularly showed 99 per cent or 100 per cent healthy germination in blotter tests. In one experiment the ''diseased'' seed consisted of the floating fraction after delinting, pregerminated for 2 days, followed by selection for appearance of fungous or bacterial infection. A second experiment was similar to the first, except that the pregermination and selection were omitted. In a third experiment the ''diseased'' seed had been inoculated with a suspension of spores of *Fusarium moniliforme* Sheld. under vacuum before planting. Each experiment consisted of 600 pots each with a ''healthy'' seed alone, 600 pots each with a ''diseased'' and a ''healthy'' seed, and 300 pots each with a ''diseased'' seed alone.

In the first experiment the ''healthy'' seed alone produced 97.3 per cent healthy seedlings, while in the presence of ''diseased'' seed the ''healthy'' seed produced 93.1 per cent healthy seedlings. In the second experiment the emergence dropped from the control value of 97.7 per cent to 95.3 per cent and in the third, from 92.0 per cent to 89.0 per cent in the presence of diseased seedlings. These results indicate that the ''diseased'' seed had only a slight and barely significant effect in lowering the germination of adjacent ''healthy'' seed, in the absence of primary infestation.

The remaining experiments, in greenhouse and field involved planting of various numbers of partially diseased seed in close contact, on the assumption that the effect of contact of a diseased seed with healthy seed would be progressively greater as the number of adjacent seeds increases, thus increasing at a logarithmic rate the opportunities for such contacts. Three experiments were performed in the greenhouse, each consisting of 203 seeds planted 1 seed per pot, 210 seeds at 2 per pot, 196 seeds at 4 per pot, 210 at 6 per pot, 224 at 8 per pot, and 210 at 10 per pot. In one test the seeds were acid-delinted without further treatment, in a second only the floating fraction of delinted seed was used, and in a third, 33 per cent of the seed had been artificially inoculated with spores of *Fusarium moniliforme* under vacuum before planting. The results of these tests are given in table 1.

In no case is there a progressive decrease in emergence with increased numbers of adjacent seeds. These experiments are consistent with the preceding experiments in indicating little or no effect of the diseased seed in lowering the emergence of adjacent healthy seedlings under rhizoctonia-free conditions.

Finally, similar observations were made in the field plantings of 1939 in comparing plantings made at the rate of 5 seeds per hill (10 possible combinations of a diseased and a healthy seed) with those at the rate of 2 seeds per hill (only 1 such combination possible). A disease-infested lot of gin-run seed was used. This was divided into 4 types of seed: nondelinted, acid-delinted but nongraded, floating fraction of delinted seed, and sinking fraction of delinted seed. Each type was planted on each of 2 planting dates in 4 50-foot rows of 60 hills at the rate of 5 seeds per hill and in 4 50-foot rows at the rate of 2 seeds per hill. In each case the percentage of emergence of the seedlings in hills of 5 was approximately equal to that of the seedlings in hills of 2. When the values:

TABLE 1.—*Contact effect of healthy and diseased seed planted in various numbers per pot*

Experiment	No. seeds per pot	Percentage of emergence
Exp. 4		
Delinted seed, not fractionated	1	80
	2	78
	4	77
	6	78
	8	78
	10	77
Exp. 5		
Delinted seed, lighter fraction	1	75
	2	70
	4	68
	6	73
	8	72
	10	70
Exp. 6		
Delinted seed, ½ inoculated with *Fusarium moniliforme*	1	73
	2	83
	4	82
	6	80
	8	84
	10	81

$$\frac{\text{Percentage of healthy emergence at 2 seeds per hill}}{\text{Percentage of healthy emergence at 5 seeds per hill}} \times 100,$$

for all types of seed, were totalled and averaged, the result was 98 per cent, indicating that the seeds planted at 5 per hill emerged at an even higher rate than those planted 2 per hill, a result slightly, but probably not significantly, in favor of the seed where the greatest number of disease contacts would have occurred. The disease-infested "light" fraction of the seed showed no greater effect of the type suggested than did the disease-free "heavy" fraction. The experiment thus confirms the preceding ones in affording no indication that the diseased seeds exert any appreciable inhibitory action on emergence of healthy seeds in the same hill.

DISCUSSION

It has been recognized by the investigators of the Cotton Disease Council that there are notable differences in the effect of Ceresan seed treatment in

Texas and Oklahoma as compared with the eastern part of the cotton belt. Each year the uniform seed-treatment tests have shown a more striking advantage with Ceresan in the eastern States, where the seed-borne *Glomerella gossypii* (South.) Edg. and *Fusarium moniliforme* are the principal agents of seedling disease; while in Oklahoma and Texas, where the soil-borne *Rhizoctonia* is the most prevalent seedling pathogen, acid-delinting of cotton seed is more advantageous than Ceresan treatment. The findings of the present study aid in interpreting this fact.

Ceresan might conceivably benefit the cotton seed either by protecting an uncontaminated seedling against infection from adjacent infested seedlings or by giving it such protection from infested soil. Since, in the foregoing experiments, no essential difference was seen between heavily infested seed, Ceresan-treated seed, and noninfested seed in the deviation from normal diseased-seedling distribution, one may conclude that the effect of Ceresan in protecting seedlings against rhizoctonia infection in neighboring seedlings is a negligible one. But, under southwestern conditions, Ceresan does increase healthy emergence, at times to a limited extent, at other times in a more striking fashion. Moreover, this beneficial effect of Ceresan is seen when the seeds to be treated are acid-delinted, already free of infestation. It is concluded that the effect of Ceresan is primarily on the seedling from the treated seed itself, and effective against either surface-seed-borne infestation or primary soil-borne infestation, but that it is not effective against plant-to-plant spread of *Rhizoctonia* in the soil. This conclusion is in agreement with that of Lehman (4) who found, as a result of trials in soil inoculated with *Rhizoctonia* that: " . . . so far as final stands of seedlings are concerned, organic mercury dust applied to cotton seed before planting may be of little, if any, value as a protectant against *Rhizoctonia* in the soil. Its protective action against fungi carried on the seed is not questioned."

There is an important need for further development of chemical means of protection of cotton and legume seed under the conditions of rhizoctonia infestation prevalent in the Southwest. A desirable fungicide for this purpose appears to be one that volatilizes over a relatively long period. However, the efficacy of such a fungicide is inversely proportional to the length of time required for seedling development, and favorable results with Ceresan-treated, acid-delinted cotton seed in rhizoctonia-infested soil indicate that the shortening of the seedling stage by delinting reduces the necessity of a long period of volatilization in the fungicide, thus increasing the efficiency of Ceresan.

In the case of seed-borne infestation, it has been seen that the infection of a seedling is not hazardous to adjacent seedlings. This has a bearing on the planting value of partially infested seed. If such a hazard did exist, seed that exhibits 80 per cent healthy germination would not be 80 per cent as productive as seed that germinates 100 per cent, since the 20 per cent of diseased seed would, in turn, lead to the destruction of numerous adjacent seedlings from the 80 per cent healthy seed, thus lowering the field germination, and, hence, the planting value to a point below 80 per cent of the pure seed. But since this hazard does not exist, the market value of seed for planting purposes may be considered proportional to its laboratory germination results, other factors being equal.

SUMMARY

A mathematical analysis was made of the survival of cotton seedlings, in field and greenhouse, under various conditions of attack by *Glomerella gossypii, Fusarium moniliforme, Rhizoctonia solani,* and other seedling-disease organisms, in efforts to determine the extent to which an infected seedling is hazardous to adjacent healthy seedlings.

With freedom from serious rhizoctonia attack, the mortality of seedlings from infested seed followed a random distribution, agreeing with the distribution formula derived by expansion of the binomial equation. A skew distribution, with an excessive number of seedling failures in hills containing one or more infested seeds, did not occur, indicating that in the absence of severe rhizoctonia attack, diseased seedlings, regardless of seed type or infestation, were not a hazard to adjacent healthy seedlings. This was confirmed by direct observation of the success of healthy seedlings in the presence of diseased ones in rhizoctonia-free soil, and also by the equal emergence rates of seedlings from mixtures of diseased and healthy seed whether planted under conditions of many or few possible contacts of diseased and healthy seedlings.

Where *Rhizoctonia* was a factor, there was an excessive proportion of seedling failures in hills originally containing one or more diseased seedlings. These failures were not due to the "all-or-nothing" character of field accidents, nor to the original internal or external infestation of the seed, but to soil-borne *Rhizoctonia* advancing in an "all-or-nothing" fashion.

These findings are in agreement with the greater usefulness of Ceresan seed treatments in the southeastern States, where seed-borne infestations are of major importance in seedling disease, and the greater success of acid-delinting of seed in the Southwest where *Rhizoctonia* is the chief cause of seedling disease, since the acid treatment shortens the period of susceptibility to *Rhizoctonia.*

The bearings of these findings on the planting value of partly infested cotton seed, and on present needs in seed treatment, are discussed.

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LITERATURE CITED

1. CHESTER, K. STARR, and G. TENNYSON. The effect of a diseased cotton seed on an adjacent healthy seed. Phytopath. (Abstract) 30: 4. 1940.
2. ————. Seed treatments for cotton. Okla. Agr. Exp. Stat. Cir. 89: 2–7. 1940.
3. HORSFALL, J. G. Combating damping-off. N. Y. Agr. Exp. Stat. (Geneva) Bull. 683. 46 pp. 1938.
4. LEHMAN, S. G. Cotton seed dusting in relation to control of seedling infection by *Rhizoctonia* in the soil. Phytopath. 30: 847–853. 1940.

A DISTINCTIVE STRAIN OF TOBACCO-MOSAIC VIRUS FROM PLANTAGO

FRANCIS O. HOLMES

(Accepted for publication April 26, 1941)

A mosaic disease was observed, in the autumn of 1940, in plants of rib grass (*Plantago lanceolata* L.) and of the broad-leaved plantain (*P. major* L.), growing as weeds in several widely separated locations near Princeton, New Jersey. Affected plants, interspersed among much larger numbers of healthy-appearing plants of the same species, were distinguished by slight twisting of petioles, chlorotic streaks along veins, and systemic chlorotic mottling (Fig. 1, A).

Inoculation of Turkish tobacco (*Nicotiana tabacum* L.) with undiluted juices expressed from affected plants of each of the *Plantago* species initiated a disease characterized by numerous primary lesions (Fig. 1, B) resembling those of tobacco-ring-spot disease. Usually these primary lesions appeared as thin, white rings of necrotic tissues surrounding green or only slightly chlorotic central areas and distributed in a random manner over the inoculated portion of the leaf lamina. Sometimes, however, they appeared as coarse, brown rings of necrotic tissues with green centers or as solid, brown necrotic spots. The primary lesions first became distinguishable on the third or fourth day after inoculation. A day or two later, comparable secondary lesions appeared in several successive uninoculated leaves at the top of each plant (Fig. 1, C). These secondary lesions consisted of rings and lines of necrotic tissues along veins; they were usually white and delicate in character but occasionally brown and coarse. Both primary and secondary necrotic lesions were sometimes zonate. Necrosis did not occur in newly formed leaves after the onset of systemic attack unless the infected plants were forced into unusually rapid growth, as by being cut back severely. Instead, the next leaves that were formed showed chlorotic mottling in which the chlorotic areas were often few and large (Fig. 1, D). As the diseased plants matured, new leaves were variously affected. Some showed a blurred chlorotic mottling; others were green and nearly normal in appearance; a few showed sharply delineated mottling in which the chlorotic areas were numerous and small.

Inoculation of *Nicotiana glutinosa* L. gave rise locally to numerous brown necrotic lesions, essentially like those produced in this host by tobacco-mosaic virus (*Marmor tabaci* H.). The lesions were slightly smaller than those characteristic of the type strain of this virus and were less heavily pigmented peripherally; no systemic spread of the disease was observed.

The two original isolates of virus, from rib-grass and broad-leaved plantain, respectively, appeared to be identical in their effects on *Nicotiana tabacum* and on *N. glutinosa*. The strain from rib grass was used in all subsequent experiments, as being representative.

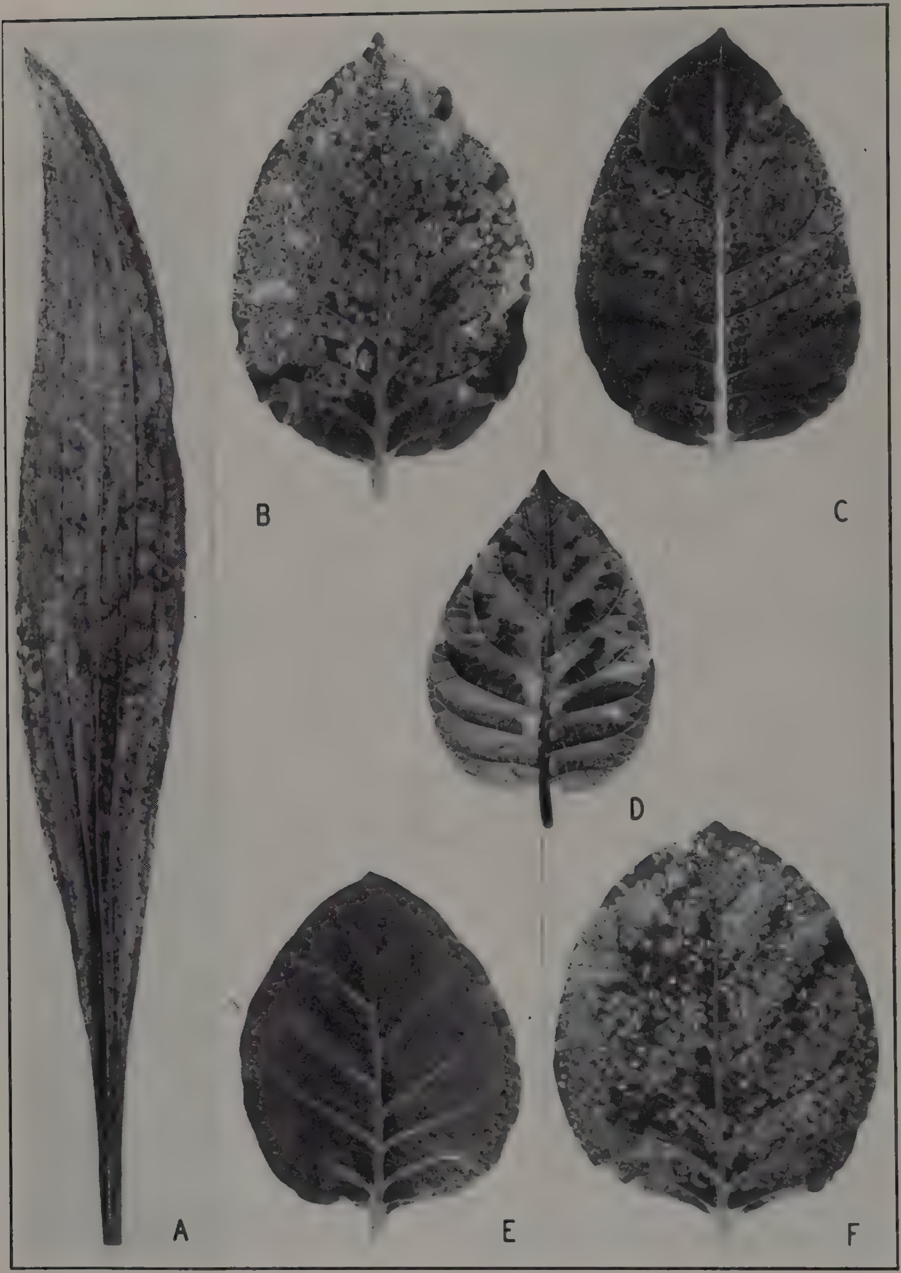

FIG. 1. Effects induced by experimental inoculation with the rib-grass strain of tobacco-mosaic virus. A. Chlorotic mottling in rib grass, *Plantago lanceolata* L. B. White necrotic-ring primary lesions in Turkish tobacco, *Nicotiana tabacum* L. C. Similar secondary lesions and, D, subsequent chlorotic mottling in Turkish tobacco. E. Absence of primary lesions in tobacco leaf protected by earlier infection with the type strain of tobacco-mosaic virus, in contrast with, F, characteristic 5-day lesions induced by the rib-grass strain in a control leaf inoculated previously only with juice of healthy tobacco and hence not protected. (Photographs by J. A. Carlile.)

The preliminary observations already described had raised the question of whether more than one virus might be concerned in the original disease in rib grass, since no single virus was known to produce all of the observed effects. Further studies were conducted, in part to answer this question and in part to permit the identification of the virus or viruses involved.

EXPERIMENTAL STUDIES OF THE RIB-GRASS VIRUS

Resistance to Heat. Preliminary tests indicated that the rib-grass virus was relatively resistant to heat. In order to appraise its stability more accurately, juice expressed from systemically infected Turkish tobacco plants, such as served as sources of inoculum for use in all subsequent experiments except as otherwise stated, was exposed in thin-walled glass tubes, without dilution, to a temperature of 85° C. for 0, 5, 10, 15, and 30-minute periods; 10-leaf tests on *Nicotiana glutinosa* gave lesion counts of 3104, 2068, 1947, 1437, and 1242, respectively, showing gradual but slight decrease of infectivity with increase of time at this temperature. Similar exposures at 90° C. gave counts of 3668, 1216, 639, 83, and 1, showing more rapid decrease of infectivity but not complete inactivation at this higher temperature. Additional tests showed that the virus was completely inactivated in 10 minutes at 93° C., not at 92° C.

Only 1 virus that has been shown to spread systemically in tobacco is known to resist exposure at 85° C. or at any higher temperatures for as long as 10 minutes without complete inactivation; this is tobacco-mosaic virus. The result of the heat treatments, in conjunction with the character of the lesions on *Nicotiana glutinosa,* gave preliminary evidence, therefore, that the rib-grass disease was caused by a strain of tobacco-mosaic virus, either alone or in mixture with some other virus. It seemed highly improbable that a second virus also could withstand this relatively high temperature treatment. Transfers were made to tobacco, therefore, from single lesions in *N. glutinosa* plants inoculated with juice that had been exposed at 85° C. for 10 minutes. These transfers induced a disease having all the characteristics of the malady earlier produced in tobacco by inoculation with crude juice taken directly from diseased rib-grass plants in the field. Thus, it appeared that all the effects observed in tobacco could be produced by a single-lesion isolate of a virus having approximately the thermal stability of typical tobacco-mosaic virus.

Resistance to Drying. A stock of the rib-grass virus in dried tobacco leaves was stored in the laboratory at about 23° C. and was tested after 73 and 122 days by inoculation of *Nicotiana tabacum* and *N. glutinosa.* A high degree of infectiousness was noted on each occasion, and the disease type was found not to have been modified by storage of the virus in dried leaf tissues. In resistance to desiccation in leaf tissues, as in resistance to heat, the rib-grass virus resembled tobacco-mosaic virus.

Filterability. A sample of juice from an infected tobacco plant was centrifuged at low speed to remove the larger particles of cellular debris; 10 cc.

of the cleared juice was then added to 85 cc. of water and 5 cc. of a 1-day-old broth culture of the red-pigment-forming bacillus, *Serratia marcescens* Bizio 1823 (=*Bacillus prodigiosus* Flügge 1886). Part of the mixture of diluted infectious juice and bacterial culture was filtered through a Berkefeld W porcelain candle; the filtrate proved to be free from bacteria, as determined by agar-plate tests of 2 samples, each of 1-cc. volume, but induced the formation of 80.2 lesions per leaf on 10 inoculated leaves of *Nicotiana glutinosa.* The filterability of the rib-grass virus was thus demonstrated. Control tests of the unfiltered mixture showed more than 1000 bacteria per cc. and a virus content sufficient to induce the formation of 64.4 lesions per leaf on 10 similar leaves of *N. glutinosa.*

Infectiousness at High Dilutions. The virus in freshly expressed juice was so concentrated that it remained infectious, even when diluted to $1:10^6$. The numbers of lesions produced on sets of 20 leaves of *Nicotiana glutinosa* inoculated with 9 successive 10-fold dilutions (10^0 to 10^{-8}) of juice expressed from a tobacco plant infected 91 days previously were: 9700, 6088, 1700, 236, 88, 14, 1, 0, 0. This ability to infect at high dilution is comparable to that of many of the more infectious strains of tobacco-mosaic virus and but little inferior to that of the type strain, though the leaves of the old plant used as source of virus had appeared green and nearly like those of healthy plants.

Inability to Infect Bean. Most strains of tobacco-mosaic virus cause the formation of brownish-red local lesions in inoculated leaves of Early Golden Cluster bean (*Phaseolus vulgaris* L.) (9), although a strain which appears incapable of producing lesions in this plant has been described by Melchers *et al.* (8). Repeated attempts to transmit the rib-grass virus to Early Golden Cluster and other varieties of bean have resulted in failure. Apparently *P. vulgaris* is immune from this virus under the conditions of the experiments. Because of this characteristic, it has been possible to show by the inoculation of bean leaves that samples of the rib-grass virus used in the tests reported here did not owe their tobacco-mosaic-like characteristics to contamination with ordinary tobacco-mosaic virus, which, if present, would have been disclosed by the development of characteristic lesions.

Effect on Nicotiana sylvestris. Dark-brown, necrotic, local lesions alone appeared on *Nicotiana sylvestris* Speg. and Comes plants that had been inoculated with the rib-grass virus. In this respect the virus resembled the so-called aucuba types of tobacco-mosaic virus, a group of substrains and isolates collectively constituting the tomato aucuba-mosaic strain of this virus (*Marmor tabaci* var. *aucuba* H.).

Response to Presence of Gene N in Tobacco. Further diagnostic evidence was obtained by direct transfer of virus from the spontaneous disease in rib grass to a segregating progeny of tobacco plants, some bearing the dominant gene *N* from *Nicotiana glutinosa,* others its recessive allele *n*. Plants bearing the dominant gene *N* respond to infection with typical tobacco-mosaic virus by forming necrotic lesions of the type characteristic of *N. glutinosa* (5), whereas plants lacking it respond by forming extremely faint chlorotic primary lesions that are sometimes difficult to detect without staining in iodine solutions (3) and by showing subsequent chlorotic mottling. The virus from rib grass also produced 2 types of disease, in some plants the brown, necrotic, local lesions of *N. glutinosa* type, and in others primary and secondary thin, white, necrotic, ring-like lesions with green or slightly chlorotic central areas, followed by mottling, as in ordinary tobacco infected with this rib-grass virus. The results of this test tended to confirm the earlier evidence that a strain of tobacco-mosaic virus was present in the diseased plants, for no other virus is known to be so influenced by the gene *N* that 2 sharply distinguishable disease types are produced. Moreover, these results confirmed the evidence already available that but 1 virus was responsible for all the observed effects. Had a second virus been responsible for the necrotic rings in ordinary tobacco, the *N*-type tobacco plants used in this experiment should have shown not only the brown necrotic spots, characteristic of infection by tobacco-mosaic virus, but also the primary and secondary ring-like lesions.

Immunological Relationships. Tobacco plants inoculated over the whole surface of a leaf with the type strain of tobacco-mosaic virus 5 days before inoculation of the same leaf with the rib-grass virus developed no necrotic lesions, whereas tobacco plants of the same age inoculated with the rib-grass strain 5 days after preliminary inoculation with juice from healthy tobacco plants developed large numbers of the characteristic necrotic lesions (Fig. 1, E and F). Furthermore, tobacco plants, systemically infected with typical tobacco-mosaic virus, did not show necrotic primary lesions as a result of subsequent inoculation with the rib-grass virus. This protection, as a result of previous infection with a strain of tobacco-mosaic virus known not to cause necrosis, confirmed the diagnosis of tobacco-mosaic virus as wholly responsible for the necrotic-ring effects in tobacco. No such protection was conferred on tobacco plants by previous infection with other mosaic viruses, such as cucumber-mosaic virus (*M. cucumeris* H.) and alfalfa-mosaic virus (*M. medicaginis* H.).

Confirmation of the protection tests in tobacco was given by experiments in which it was demonstrated that protection was afforded to parts of leaves of *Nicotiana sylvestris* by inoculations with typical tobacco-mosaic virus 5 days before their inoculation with the rib-grass virus. No necrotic lesions appeared in areas already invaded by the type virus, but many necrotic lesions appeared in uninoculated areas or in areas rubbed only with juice of healthy tobacco in the preliminary inoculation. Kunkel (6) has shown that this type of test is an excellent criterion for determining the possible relationship of a virus to known strains of tobacco-mosaic virus.

Serological Relationships. Characteristic flocculent precipitates were secured with the rib-grass virus in the presence of antiserum to the type strain of tobacco-mosaic virus. The samples of rib-grass-mosaic virus used in this serological test were shown to be highly infective, typical, and free from contamination with ordinary tobacco-mosaic virus by inoculation of

Nicotiana glutinosa, N. tabacum, and *Phaseolus vulgaris.* Large numbers of lesions in the first of these species reflected the high titer of the sample in hand, necrotic-ring production in the second showed the nature of the strain to be unmodified, and complete lack of lesions in the third showed the absence of ordinary tobacco-mosaic virus, control inoculations with which produced abundant reddish necrotic lesions, as usual, in accompanying bean plants grown simultaneously and maintained under the same conditions in the greenhouse.

Morphology. Electron micrographs, made by Thomas F. Anderson, Fellow of the National Research Council at the laboratories of the Radio Corporation of America in Camden, N. J., showed the rib-grass-virus particles to be rod-like in form. A portion of one of these micrographs is represented in figure 2, with a scale superposed on it to facilitate estimation of particle lengths. Preparations of the rib-grass virus that had been purified by ultracentrifugation were examined by I. Fankuchen, National Research Council Fellow in Protein Chemistry at the Physics Department,

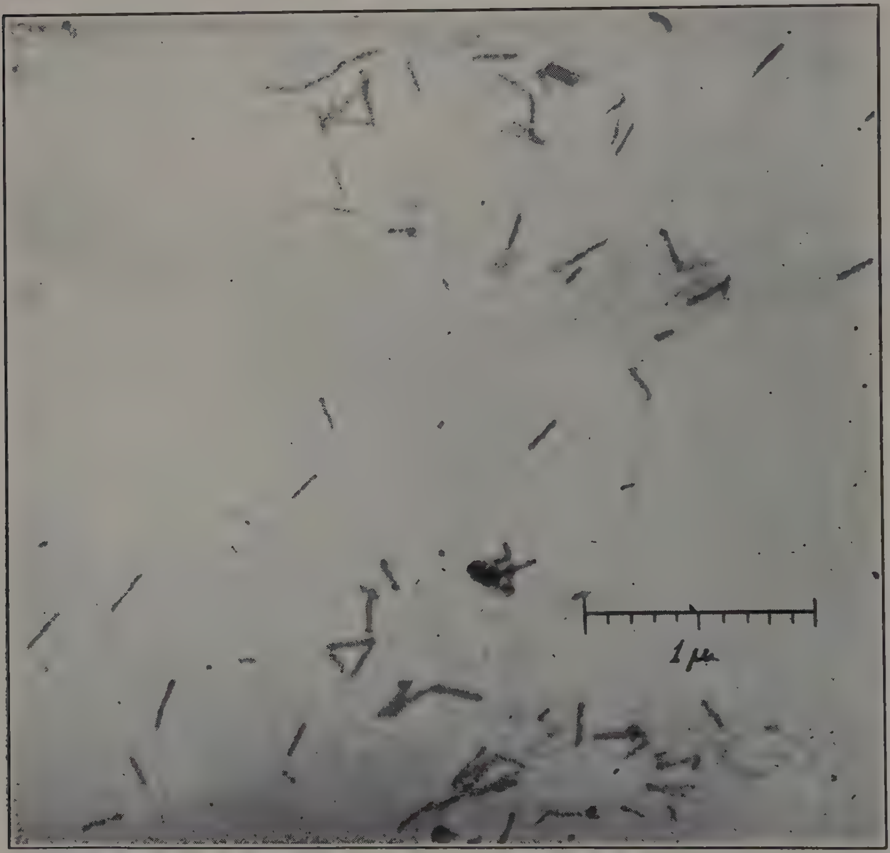

FIG. 2. Electron micrograph of the rib-grass strain of tobacco-mosaic virus reproduced at a magnification of 30,000 diameters. (Micrograph by T. F. Anderson.)

Massachusetts Institute of Technology. Through the use of an X-ray diffraction technique (2), he determined the average diameter of the virus particles to be about 15.0 mμ (150 Å.) in air-dried preparations. Both the rod-like shape disclosed by use of the electron microscope and the width of particles as measured by X-ray methods agreed well with the hypothesis that the rib-grass virus was a strain of tobacco-mosaic virus.

Invasiveness. The rib-grass virus differed from the type strain of tobacco-mosaic virus in its greater ability to invade plants of *Plantago lanceolata* systemically, the type strain being known to increase in the inoculated leaf (4, p. 64), but not to produce obvious disease, and not to spread to new leaves in this host; indeed, Allard (1, p. 11) reported that the most rigorous methods of inoculation were ineffective in producing any visible symptoms of disease in rib grass. Perhaps it is not surprising, therefore, that the rib-grass strain was found less able to penetrate the tissues of some solanaceous species, its specialization for *Plantago* being accompanied by a notable reduction of invasiveness in such plants as tomato and *Physalis angulata* L.

In tomato (*Lycopersicon esculentum* Mill.), the rib-grass virus produced either localized and inapparent infections, or, less often, a systemic disease characterized by few secondary lesions, constituting a spotty chlorotic mottling. Typical tobacco-mosaic virus under the same environmental conditions regularly became systemic and caused a well-developed chlorotic mottling in this host.

In *Physalis angulata,* only primary lesions were formed by the rib-grass virus; these were mostly chlorotic in character, though a few showed necrotic peripheries; secondary lesions were absent and no defoliation occurred. Typical tobacco-mosaic virus became systemic in this host; its primary and secondary lesions were nearly all in the form of fine white necrotic rings with green centers, a few being coarser necrotic rings or necrotic brown spots; abscission of affected leaves led eventually to complete defoliation of the plants.

Inability to Infect Cucumber. Despite the ability of the rib-grass virus to invade *Plantago* systemically, an ability not possessed by typical tobacco-mosaic virus, cucumber (*Cucumis sativus* L.) appeared to be insusceptible to infection, no disease appearing after inoculation and no evidence of virus increase being obtained on subinoculation. In this inability to infect cucumber, the rib-grass virus resembles typical tobacco-mosaic virus and differs sharply from cucurbit-mosaic virus (*Marmor astrictum* H.), which has no known hosts in common with tobacco-mosaic virus but shares its physical and chemical properties, even to the point of antigenic similarity.

Stability. The rib-grass virus is relatively stable, at least in the sense that it has not been observed to give off variants resembling ordinary tobacco-mosaic virus. Tobacco plants, infected with the rib-grass virus when young and tested more than 2 months later, showed no evidence of the development of strains of the virus capable of infecting bean plants. Ten successive transfers in tobacco failed to modify the virus enough to permit it to form lesions in the bean, to move freely in tomato, or to mottle tobacco without inducing the formation of necrotic-ring lesions. Tomato plants, infected when young, usually showed no evidence of disease to blossoming time; in a few plants there were scattered chlorotic lesions in upper leaves. Inoculated leaves of tomato plants contained much virus when tested 18 days after inoculation; the tops of the same plants, however, showed no virus in some cases and but little in others; this would not have been the case had variants resembling the type strain been formed.

Existence of Substrains. Successive isolates from diseased rib-grass plants in the field disclosed the existence of variants of the rib-grass virus. All isolates, however, produced necrotic primary lesions on transfer to Turkish tobacco plants. All were similar in being uninfectious for the Early Golden Cluster bean. One isolate was characterized by its tendency to produce necrotic rings on Turkish tobacco a little later than the others. On the whole, the similarities between the isolates were more striking than the slight differences that were detected. Because of this, the isolates thus far observed seem to constitute a closely related group and collectively to represent a strain of tobacco-mosaic virus, adapted to *Plantago* as a natural host, characterized by producing ring-like necrotic primary lesions in Turkish tobacco, and typified by the isolate described in detail in this paper.

DISCUSSION

Similarity to Recognized Strains of Tobacco-mosaic Virus. The rib-grass virus resembles to some extent a tomato-mosaic strain of tobacco-mosaic virus studied by Melchers *et al.* (8); this strain also failed to infect bean and produced only necrotic local lesions on *Nicotiana sylvestris;* it differed by producing no obvious primary lesions of any sort and no secondary necrotic-ring lesions, but only chlorotic mottling, in "Samsun" tobacco, a variety much like the Turkish tobacco used in the present study. In at least one way, the rib-grass virus resembles also the mild dark-green mosaic virus of McKinney (7, pp. 565–566), which is possibly a strain of tobacco-mosaic virus. This virus is said not to infect tomato, and, for its part, the rib-grass strain is not easily induced to move systemically in the tomato nor to produce outward manifestations of disease in this host. The mild dark-green mosaic virus of McKinney, however, like the tomato-mosaic virus of Melchers *et al.*, does not produce necrotic-ring lesions in tobacco. Resemblance of the rib-grass virus to the tomato aucuba-mosaic strain of tobacco-mosaic virus in production of localized necrosis in *N. sylvestris* is also of but limited significance, for previously recognized, *aucuba*-type substrains cause no necrotic, but only chlorotic, effects in Turkish tobacco and readily infect the bean. The minute-lesion strain of tobacco-mosaic virus (*Marmor tabaci* var. *artum* H.), which produces even smaller lesions in *N. glutinosa* than does the rib-grass strain, causes no necrosis in Turkish tobacco and infects the bean, though its lesions tend to be small in this host as in *N. glutinosa.* On the whole, the rib-grass virus shows striking characteristics that differentiate it from previously recognized strains.

Technical Designation of the Rib-grass Strain of Tobacco-mosaic Virus. The rib-grass virus appears to deserve consideration as a distinctive strain of tobacco-mosaic virus, its identity with this virus being clearly shown by its remarkable resistance to heat and desiccation, its precipitation by an appropriate antiserum, its exclusion from tissues previously infected by typical tobacco-mosaic virus but not by other viruses, and its response to the genic constitution of tobacco. The rib-grass strain of tobacco-mosaic virus is unusual in its adaptation to rib grass, in its tendency to induce peculiar necrotic-ring effects in tobacco, and in its inability to infect the bean. In particular, the induction of characteristic necrotic-ring effects in Turkish tobacco would appear to warrant its recognition as an entity deserving varietal status, occurring in nature, consisting of closely related substrains, and typified by the isolate here described. The name **Marmor tabaci** var. **plantaginis** n. var. is hereby given to it.

There has been some adverse criticism of the application of varietal names to strains of viruses (10). This criticism appears to be based on the belief that later collections may not exactly duplicate an isolate first described as type of a variety. Would not this objection apply also to the naming of a species? Minor variations among subsequent isolates, however, if only of subsidiary character and significance, need not interfere with assignment of an isolate to an otherwise appropriate category, whether of specific, varietal, or other rank.

SUMMARY

A filterable virus, causing a mosaic disease of rib grass, *Plantago lanceolata* L., and of the broad-leaved plantain, *P. major* L. (*Plantaginaceae*), was found to produce necrotic-ring patterns when transferred experimentally to Turkish tobacco (*Nicotiana tabacum* L.). Its failure to be inactivated by heat when exposed at 92° C. for 10 minutes, its inability to form its characteristic necrotic primary lesions in tissues of *N. tabacum* and of *N. sylvestris* Speg. and Comes, already invaded by typical tobacco-mosaic virus, its precipitation by tobacco-mosaic-virus antiserum, and its response to the genic constitution of tobacco showed that it was a strain of tobacco-mosaic virus (*Marmor tabaci* H.). This rib-grass strain of tobacco-mosaic virus, distinguished from previously known strains by its ability to form necrotic-ring lesions in tobacco, was given the varietal designation *Marmor tabaci* var. *plantaginis* n. var.

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LITERATURE CITED

1. ALLARD, H. A. The mosaic disease of tobacco. U. S. Dept. Agr. Bull. 40. 1914.
2. BERNAL, J. D., and I. FANKUCHEN. Jour. Gen. Physiol. 25: 111–165. 1941.
3. HOLMES, F. O. Local lesions of mosaic in Nicotiana tabacum L. Contrib. Boyce Thompson Inst. 3: 163–172. 1931.
4. ————. Taxonomic relationships of plants susceptible to infection by tobacco-mosaic virus. Phytopath. 28: 58–66. 1938.
5. ————. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopath. 28: 553–561. 1938.
6. KUNKEL, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. 24: 437–466. 1934.
7. MCKINNEY, H. H. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. Jour. Agr. Res. [U. S.] 39: 557–578. 1929.
8. MELCHERS, G., G. SCHRAMM, H. TRURNIT, and H. FRIEDRICH-FREKSA. Die biologische, chemische und elektronenmikroskopische Untersuchung eines Mosaikvirus aus Tomaten. Biol. Zentralb. 60: 524–556. 1940.
9. PRICE, W. C. Local lesions on bean leaves inoculated with tobacco mosaic virus. Am. Jour. Bot. 17: 694–702. 1930.
10. VALLEAU, W. D. Classification and nomenclature of tobacco viruses. Phytopath. 30: 820–830. 1940.

THE REACTION OF COTTON VARIETIES TO FUSARIUM WILT AND ROOT-KNOT NEMATODE[1]

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The importance of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, in increasing the severity of fusarium wilt of cotton, cowpeas, and some other crops has long been recognized. Young (10) has reviewed the more pertinent literature concerning cotton wilt and root knot prior to 1928. Recently the presence of the meadow nematode, *Pratylenchus pratensis* (de Man) Filipjev, in wilt soils, has been reported to bring about a more severe wilt condition in Georgia (1) and North Carolina (9) than has been attributed to severe infestations of the root-knot nematode alone. Several other species of nematode have recently been found parasitic on the roots of cotton plants in Georgia (2). Pathologists and plant breeders generally have directed their attention to studies of the wilt pathogen and to breeding for fusarium-wilt resistance, largely ignoring the nematode problem. It appears important to give more consideration to the nematode problems, particularly in breeding for wilt resistance in cotton. While it is recognized that the data presented in this paper are inadequate for fully establishing the relative root knot-wilt resistance of the more important commercial varieties of the southeastern States, the information on this subject is so meager and the importance of the problem is such that this preliminary note may be justified.

King and Loomis (6) have pointed out the greater resistance of upland Acala cotton to root knot in comparison with American Egyptian (Pima) grown in Arizona. Miles (7) conducted studies in Mississippi in which he used the percentage of plants showing root-knot galls as a means of differentiating varieties for root-knot resistance. Upland wilt-resistant varieties as a group were found to be more resistant to root knot than wilt-susceptible varieties. Some exotic varieties and hybrids were clearly more root-knot-susceptible than the upland cottons.

VARIETIES AND METHOD OF RECORDING DATA

The varieties studied by the writer were grown in the Coastal Plain area of Georgia in 1940 at 3 locations in 4 tests on soils known to be infested with root-knot nematode and the wilt organism, *Fusarium vasinfectum* Atk. From identical plantings of 8 varieties, grown near Hawkinsville and Plains, 4 of the 8 replicates were examined plant by plant for root-knot infestation. Like data on nematode infestation were recorded by examining all plants in two replications in each of two identical 15-variety tests located near

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[1] Cooperative investigations of the Georgia Agricultural Experiment Station and the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Approved by the Director of the Georgia Agricultural Experiment Station as Technical Contribution 80.

Hawkinsville and Americus. The data obtained from the two 15-variety tests were combined to give the mean root-knot reaction of plants from 4 plots 100 ft. long. Yield and wilt data were recorded for all 8 replicates in each of the 4 variety tests.

Root-knot-infestation examinations also were made at Plains of Seabrook (Sea Island) and Half and Half varieties and their hybrids. The plantings were made in consecutive 25-foot rows in the following order: one row of

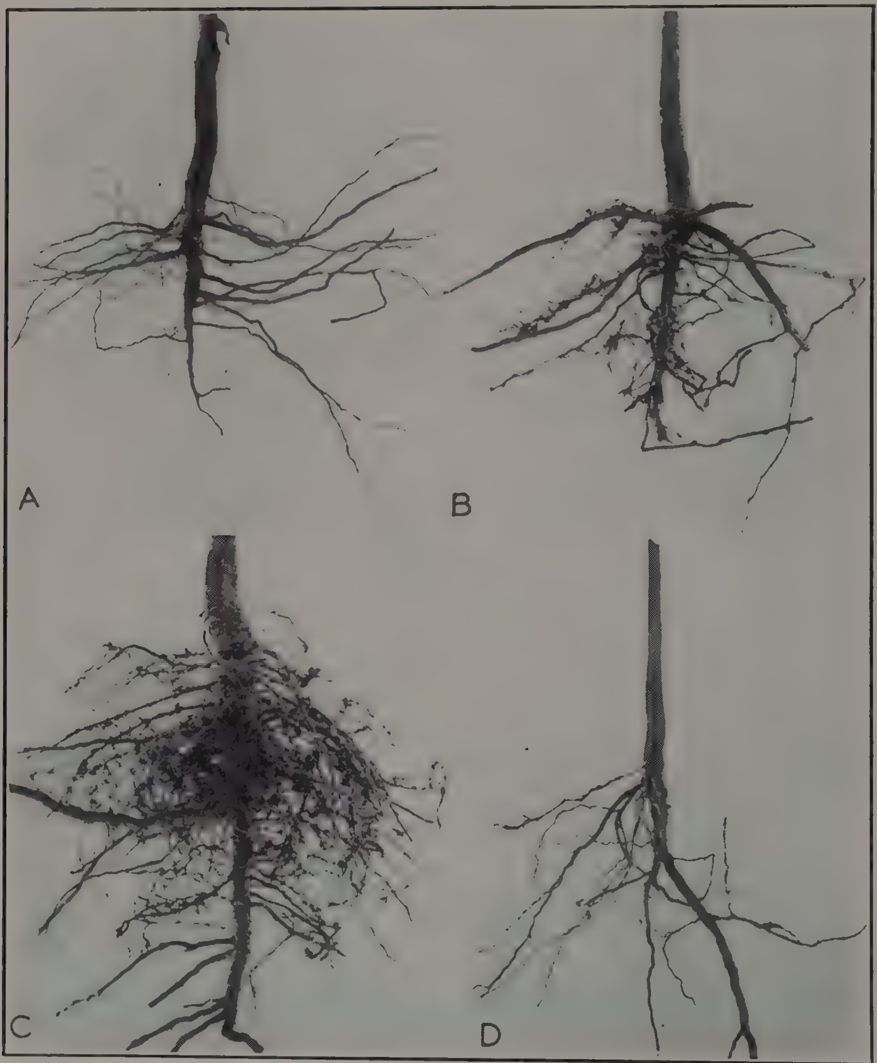

FIG. 1. A. Root of Stoneville 2B plant free from root-knot galls showing taproot and relatively sparse number of lateral roots. B. Numerous galls on root of the same variety. C. Root of Seabrook 36–12–B2, a Sea Island variety, with a mass of short lateral roots all with numerous galls (compare with D). D. A Half and Half root from a nearby planting. The development of a mass of short lateral roots as in B and C is characteristic of most upland roots in class 4.

Sea Island, Seabrook 36–12–B2, 10 rows of Sea Island, Seabrook 36–12–B2 × Half and Half, F_2, and one row of Half and Half. Three plants of each of the varieties mentioned were used in the original cross. Since the populations were small, only the mean root-knot infestation is presented for the hybrid progenies. The wilt infestation was mild in the area where this planting was made, and wilt data were not recorded.

The evaluation of root-knot reaction requires a method of distinguishing wide differences in degrees of infestation between individual plants. Preliminary observations indicated extreme variation within and between varieties. The tap root of cotton with the relatively sparse number of lateral roots (Fig. 1, A) permits a rapid observation of nematode galls. It was found practical to determine the percentage of lateral roots originating at the tap root, which showed galls. This was done in the beginning by actual count and later by estimation. The percentages were then classified and designated by numbers 1 to 4, as follows: class 1, 1 to 25 per cent; class 2, 26 to 50 per cent; class 3, 51 to 75 per cent; and class 4, 76 to 100 per cent. Plants with only the tap root infested were placed in class 1. Those without galls were classified as 0. On completion of cotton picking, nematode infestation was determined from roots that had been plowed up and stored under cool, humid conditions until the examination was completed.

Wilt data were recorded 3 times during the growing season. The final wilt percentage includes plants killed throughout the season plus additional plants showing external wilt symptoms on the last date of recording data.

RESULTS

The differentiation of varieties for root knot reaction was somewhat more striking in the Plains study (Table 1). All plants of two varieties, Stoneville 2B and Miller 610, bore root-knot galls, which indicated a uniformly heavy root-knot population in the soil. The wilt infestation was mild and consequently relatively few wilt-killed plants were necessarily discarded.

In the Plains nursery a comparison of the percentages of plants of Miller 610 and Early Wilt occurring in the different infestation classes indicates that these varieties are quite distinct as to resistance to root knot. Approximately 78 per cent of the Early Wilt plants are in classes 0, 1, and 2, compared to 8 per cent of those of Miller 610. Less than 10 per cent of the 3 root-knot-resistant varieties in the Plains nursery were in class 4, whereas of the 4 root-knot-susceptible varieties 65 to 80 per cent of the plants were in class 4. In figure 2, the plants from a single 100-foot row of Early Wilt are classified for root knot reaction and compared to an adjacent row of Miller 610 (A and B). The varieties Cleveland and Stoneville 2B, also, are compared (C and D). Plants in class 4 commonly have a mass of short lateral roots, all bearing numerous knots definitely restricted to a narrow area about the tap root (Fig. 1, B and C). The number of galls per plant in the infestation classes increases directly with the percentage of roots infested. The number of galls per plant in class 1 varied from 1 to seldom

TABLE 1.—*Numerical evaluation of root-knot-nematode infestation, yield and wilt of cotton varieties and hybrids. Plains, Hawkinsville, and Americus, Georgia, 1940*

Variety	Plants examined	Fusarium-killed and wilted plants	Plants in root-knot class					Yield of seed cotton per acre
			0	1	2	3	4	
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Pounds*
Plains variety test								
Early Wilt	371	0.6	17.9	38.2	21.8	14.4	7.9	824
4 in 1, strain 4	372	2.3	16.1	31.8	29.3	14.4	8.9	858
Cleveland (Wannamaker)	366	0.7	11.8	25.6	27.7	28.0	6.9	897
Half and Half	393	43.9	4.0	13.0	23.8	31.7	27.5	673
Stoneville 2B	360	23.3	0.0	4.4	12.0	18.1	65.5	863
Dixie Triumph 06–366	368	3.4	1.3	6.5	6.7	15.1	70.6	829
Delfos 425	429	4.6	0.8	1.2	4.4	17.9	75.7	656
Miller 610	429	14.8	0.0	1.9	5.8	12.4	80.4	738
Hawkinsville variety test								
Early Wilt	462	2.5	40.9	36.8	10.7	7.0	4.7	1,393
4 in 1, strain 4	507	6.5	44.9	33.1	10.2	7.4	4.5	1,625
Cleveland (Wannamaker)	393	2.5	55.2	29.6	9.6	4.7	1.1	1,175
Half and Half[a]	183	67.4	54.3	25.2	8.6	8.6	3.2	713
Stoneville 2B	438	21.5	32.1	17.4	14.3	14.5	21.8	1,497
Dixie Triumph 06–366	413	6.8	16.7	15.9	11.1	16.7	39.5	1,210
Delfos 425	493	9.4	6.0	16.0	15.2	19.9	42.9	1,182
Miller 610	386	18.4	12.1	11.9	11.1	15.8	49.1	1,283
Americus and Hawkinsville 15 variety tests (data combined)								
Early Wilt	518	0.9	56.9	31.9	5.9	3.6	1.6	1,297
4 in 1, strain 4	349	3.0	55.1	30.0	5.9	6.4	2.5	1,171
4 in 1, strain 3	167	6.1	39.2	34.2	10.5	8.7	7.4	1,293
Clevewilt 7–2	497	3.0	25.6	23.1	12.8	14.7	23.7	1,345
Cleveland 6 (Wannamaker)	399	3.8	33.9	32.8	14.1	11.5	7.7	1,321
Cook (Rhyne)	562	1.2	43.0	22.9	12.6	10.9	10.5	1,308
Toole (Perry)	363	2.3	28.4	17.7	12.5	15.2	26.1	1,067
Dixie Triumph 12	98	2.0	8.2	15.3	19.4	22.4	34.7	1,126
Dixie Triumph 9	249	5.1	13.9	20.7	12.8	13.3	39.3	1,110
Stoneville 2B	591	14.4	10.1	16.6	14.1	17.5	41.7	1,288
Deltapine 12	359	9.5	15.9	6.6	8.9	11.1	57.4	1,094
Coker 100–3[a]	125	47.2	53.9	10.1	9.5	7.1	19.4	807
Coker 200–2[a]	98	47.6	31.9	11.2	15.1	17.0	24.6	785
Piedmont Cleveland	289	15.5	16.9	10.7	11.4	15.0	45.8	1,145
Hibred	278	28.5	16.8	20.9	12.1	16.3	33.9	733
Sea Island × Half and Half test at Plains								
Seabrook 36–12B–2	32	[b]	0.0	15.6	3.1	0.0	81.2	....
Seabrook 36–12–B–2 × Half and Half, F_2	205	....	1.5	3.9	5.4	8.3	81.0	....
Half and Half	16	[b]	0.0	31.2	37.5	6.2	25.0	....

[a] Root-knot data are not entirely comparable; a high percentage of wilt-killed plants and additional plants with decayed roots were necessarily discarded.

[b] Wilt reaction of Half and Half shown in Plains and Hawkinsville tests above; Seabrook is considered immune from wilt.

more than 10. The galls per plant in class 4 were often 50 to 100 or more. Differences in size of galls were quite noticeable for the different varieties and class groups. Miller 610 and Stoneville (Fig. 1, B) roots show unusually large galls. Galls occurred on primary lateral roots of considerable size, whereas with roots of resistant varieties in the lower infestation classes, the galls were generally much smaller and confined to smaller roots, a condition suggesting an actual qualitative difference in response of root tissues to root-knot-nematode infection.

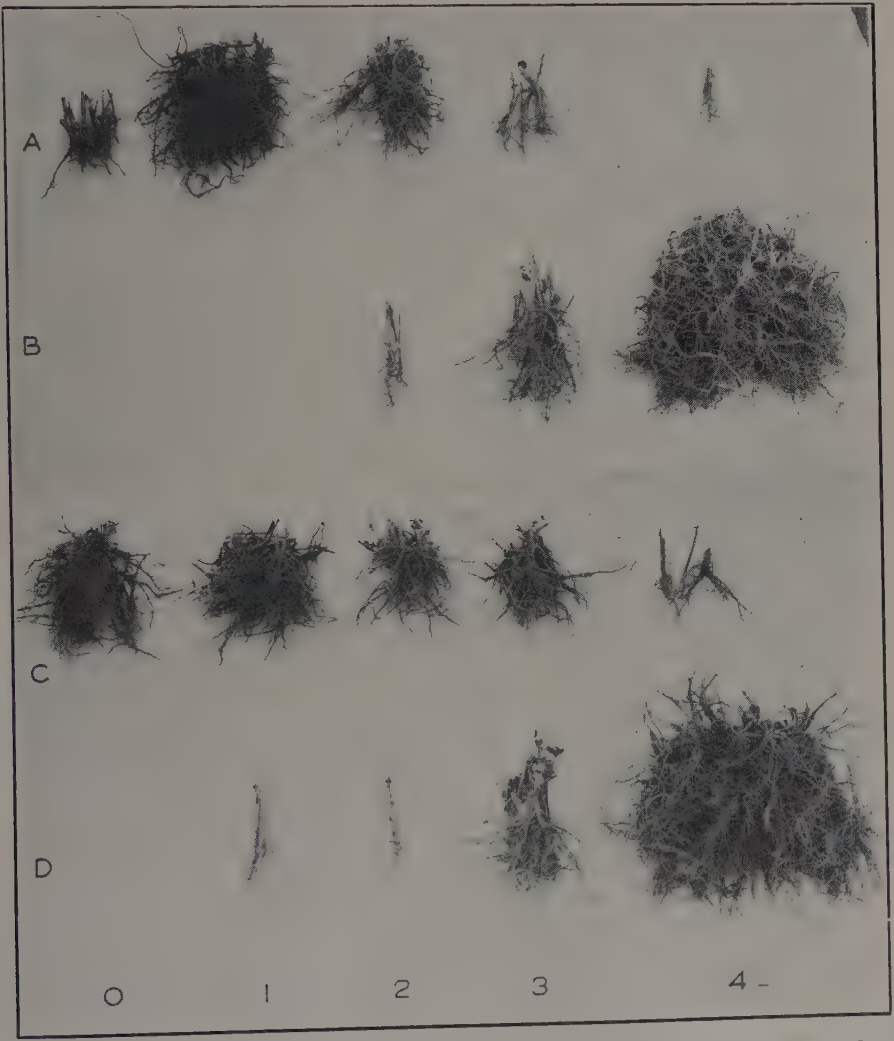

FIG. 2. Roots of cotton plants from single 100-foot plots evaluated for root-knot infestation on a basis of percentage of lateral roots showing galls, percentages grouped as follows: 0, not infested; 1, 1 to 25 per cent infested; 2, 26 to 50 per cent infested; 3, 51 to 75 per cent infested; and 4, 76 to 100 per cent infested. A. Early Wilt; B. adjacent row of Miller 610; C. Cleveland (Wannamaker), and D. Stoneville 2B.

A graphic illustration of the reaction to root knot in the case of 8 varieties grown in the Plains nursery further indicates the wide differences in resistance (Fig. 3, A). The varieties Early Wilt, Coker's 4 in 1 strain 4, and Cleveland, compared to Stoneville 2B, Dixie Triumph 06–366, Delfos 425, and Miller 610, are highly resistant. The mean reaction (Fig. 3, B) of the former group, all developed by breeders in the Coastal Plain area of South Carolina, on wilt soils probably heavily infested with root knot nematode, is quite different from the mean reaction of the latter group, originated in the heavier and more sparsely root-knot-infested soils of Louisiana and Mississippi. Half and Half, originated in the Piedmont region of Georgia, is intermediate in type. Approximately 30 per cent of the Half and Half roots were discarded because of wilt and decay, and the data may not be so trustworthy as they are for other varieties. In the Hawkinsville study the root-knot symptoms were somewhat less severe than in the study at Plains, a fact evidenced by the higher percentage of plants in all varieties placed in the noninfested class. Although the infestation was lighter, the varieties maintain their same relative positions in resistance to root knot, with the exception of Half and Half. The plants examined in this variety represented only 24 per cent of the original stand, the remainder having been

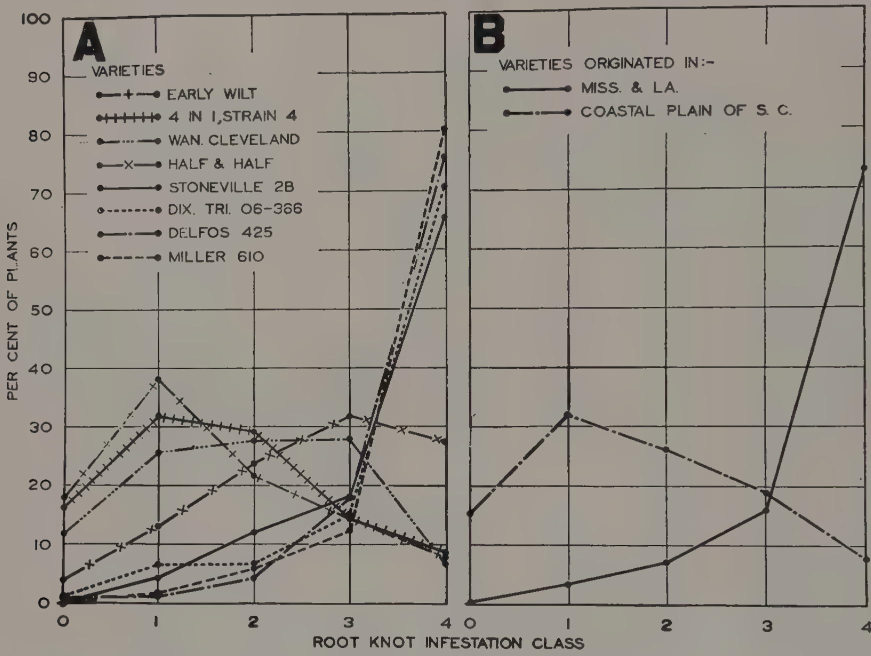

FIG. 3. Eight varieties of cotton grown near Plains, Georgia, in 1940 evaluated for root-knot infestation (A) individually and (B) grouped to show the place of varietal origin effect on root-knot susceptibility. Early Wilt, 4 in 1 strain 4, and Wannamaker Cleveland were originated in Coastal Plain soils, where root-knot infestation is severe. The varieties Stoneville 2B, Dixie Triumph 06–366, Delfos 425, and Miller 610 were originated in the heavier soils of Mississippi and Louisiana. Half and Half, developed in the Piedmont, is not included in either group.

killed by wilt or otherwise discarded because of decayed roots; hence, the final root-knot data are obviously inaccurate. There is further agreement in the relative root-knot resistance of varieties occurring in the 15-variety test (Americus and Hawkinsville combined) in comparison with the Plains and Hawkinsville studies. This can be seen by observing the reaction of Early Wilt, Coker's 4-in-1 strain 4, and Stoneville 2B, varieties that occur in all 3 tests.

A comparison of the root-knot symptoms of Half and Half and Seabrook showed quite noticeable differences. Typical class 4 plants of each variety are shown in figure 1, C and D. Seabrook appeared highly susceptible in comparison with the intermediate reaction of Half and Half. The mean reaction of the 10 F_2 hybrid progenies was rather like that of the more susceptible parent. Wilt data were not recorded for this study, but Half and Half is considered entirely susceptible to, whereas Seabrook for all practical purposes is considered immune from wilt. *Fusarium vasinfectum*[2] has been cultured from discolored vascular tissue of Seabrook, but external wilt symptoms have not been observed in the field.

A consideration of the wilt reaction of all the varieties and their reaction to root knot indicates, in general, a negative relationship. High root-knot resistance, in the writer's experience, is confined to varieties selected for wilt resistance. On the other hand, Dixie Triumph 06–366, Delfos 425, and Sea Island Seabrook 31–12B–2 are high in wilt resistance but are rather highly susceptible to root knot. The most wilt-susceptible variety (Half and Half) shows intermediate resistance to root knot. The varieties Miller 610, Stoneville 2B, Deltapine 12, and Piedmont Cleveland, intermediate in wilt resistance, are apparently susceptible to root knot. Many plants with decayed root systems occurred in all root-knot-susceptible varieties in contrast to root-knot-resistant varieties. King and Hope (5), in Arizona, made similar observations in which the roots of the highly root-knot-susceptible Pima variety were largely decayed, while those of the more nematode-resistant upland Acala remained alive.

A comparison of the yield, wilt, and root-knot reaction data indicates the superiority of the wilt- and nematode-resistant varieties as a group. The low yields of such varieties as Coker 100–3, Coker 200–2, and Hi-Bred indicate the effect of susceptibility to wilt on yield. It is clearly not possible to segregate the wilt and root-knot effects on yield in the southeastern States, where the soils generally are infested with both pathogens. It is logical to assume that some reduction in yield would result from the occurrence of large numbers of plants in a variety with root-knot conditions as shown in figure 1, B and C. King *et al.* (4) in limited studies, on root-knot-free and heavily infested soils, found the yield of upland Acala to be unaffected. Godfrey (3), in an intensive study of the pineapple root system as affected by the root-knot nematode, describes a situation that appears applicable to the cotton plant. He found a common condition in which the primary roots escaped serious injury and the plants became well-established, permitting good initial root and plant growth and good plant-crop yield. Also, in later stages of plant growth, he found that heavy infections may occur as the result of the gradual building up of a high nematode population from primary infections in branch rootlets and small roots resulting in the common condition of long main roots relatively free from infection, and new, short, heavily infected axillary roots. From the experience of numerous investigators (10) considerable crop loss from root knot results from the planting of wilt-susceptible or moderately susceptible varieties of cotton on wilt- and nematode-infested soils. The resultant yield losses can be partly attributed to the effect of the root-knot nematode in increasing the wilt infection.

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[2] Personal communication with G. M. Armstrong.

DISCUSSION

The writer's experience indicates that resistance to root knot has been established only in those varieties selected for wilt resistance in Coastal Plain soils. Susceptibility to root knot presumably increased susceptibility to wilt in segregating progenies; and such lines were eliminated in the process of breeding for wilt resistance. The writer is not aware that any of the originators of root-knot-resistant varieties have given direct attention to this problem; consequently, it is assumed that such root-knot resistance as occurs was incidental and resulted through selection for wilt resistance on soils infested with both parasites.

The greatest promise of obtaining higher yielding, wilt-resistant varieties of cotton at present involves the hybridizing of wilt-resistant types with the earlier, higher-yielding varieties, such as Stoneville 2B, Miller, Deltapine, and others. Thus it appears that the introduction of susceptibility factors for both wilt and root knot will considerably reduce the probability of quickly recovering wilt-resistant types. The rate at which backcrosses can be made to the wilt- and root-knot-susceptible parent will depend upon the speed with which resistance to these two factors can be reestablished in the segregating progenies. The relationship of root-knot resistance to wilt resistance suggests the importance of giving direct attention to the root-knot reaction of segregating material, and the management of breeding plots to insure maintenance of a severe root-knot infestation in combination with an abundance of wilt inoculum. It was pointed out that a fairly high degree of wilt resistance can be established in the absence of root-knot resistance, *e.g.*, Delfos 425 and Dixie Triumph 06–366. However, it appears of particular significance that the currently most promising wilt-resistant varieties for Coastal Plain soils are those that combine high resistance to both parasites.

In summarizing the results of a regional study of cotton varieties grown on wilt soils it was concluded (8) that "relative differences in varietal susceptibility to wilt at different locations appears to be partly attributable to varying nematode infestations and differences in varietal reaction to a combination of wilt and nematodes." The evidence of wide varietal differences in susceptibility to root knot reported in this paper further substantiates this conclusion.

SUMMARY

Some preliminary observations are presented to show that the more important cotton varieties currently planted in the southeastern United States vary widely in root-knot resistance. Resistance to root knot appears associated with wilt resistance; but it is suggested that this is incidental, since some wilt-resistant varieties show no more root-knot resistance than wilt-susceptible and semi-resistant varieties.

Resistance to root knot was confined to wilt-resistant varieties that were originated in lighter Coastal Plain soils, whereas none of the wilt-resistant varieties originating in the heavier sparsely root-knot-nematode infested soils of Mississippi and Louisiana showed root-knot resistance.

Varieties combining root-knot and wilt resistance are required for Coastal Plain conditions. These observations indicate that, while such combined resistance has been secured in some cases without conscious effort in breeding for root-knot resistance it may be highly important for the breeder and pathologist to devote some attention to root-knot resistance.

A system of numerical evaluation of root-knot infestation in cotton plants is described showing that it is possible to classify increasing root-knot severity by increasing numbers, with 0 representing plants void of root-knot galls and 1 to 4 representing percentages of roots infested at increments of 25 per cent.

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LITERATURE CITED

1. ANONYMOUS. The meadow nematode, *Pratylenchus pratensis* (de Man) Filipjev, identified on cotton roots in Georgia in 1937. Ga. Agr. Exp. Stat. 50th Ann. Rpt. **20**. 1937–38.
2. ———. New nematode species parasitic on cotton roots. Ga. Agr. Exp. Stat. 52nd Ann. Rpt. **16**. 1939–40.
3. GODFREY, G. H. The pineapple root system as affected by the root-knot nematode. Phytopath. **26**: 408–428. 1936.
4. KING, C. J., R. E. BECKETT, and ORLAN PARKER. Agricultural investigations at the United States Field Station, Sacaton, Arizona, 1931–35. U. S. Dept. Agr. Cir. 479. 1938.
5. ———, and CLAUDE HOPE. Field practices affecting the control of cotton root knot in Arizona. U. S. Dept. Agr. Cir. 337. 1934.
6. ———, and H. F. LOOMIS. Agricultural investigations at the United States Field Station, Sacaton, Ariz., 1925–30. U. S. Dept. Agr. Cir. 206: 17–20. 1932.
7. MILES, L. E. Some tests of varietal susceptibility to a combination of root-knot nematode and cotton wilt. Phytopath. **29**: 974–978. 1939.
8. SMITH, A. L. A regional study of the relationship of potash treatments to the development of cotton wilt under widely varying conditions of soil and environment. Phytopath. (Abstract) **30**: 707. 1940.
9. TAYLOR, A. L., H. D. BARKER, and O. P. OWENS. Preliminary report on cotton wilt-nematode experiments at Lumberton, N. C. Phytopath. (Abstract) **29**: 752. 1939.
10. YOUNG, V. H. Cotton wilt studies. I. Ark. Agr. Exp. Stat. Bull. 22[illegible] 1928.

THE SPORE-GERMINATION METHOD OF EVALUATING FUNGICIDES[1]

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Of the methods that have been developed for testing protective fungicides in the laboratory, the spore-germination method, outlined in 1910 by Reddick and Wallace (7), and further developed by McCallan (3), is now, undoubtedly, the one most widely used. In McCallan's procedure fungous spores are dispersed in an aqueous suspension or solution of the toxic agent and pipetted as drops onto chemically clean glass slides, or the slides may be sprayed with toxic agent and the aqueous suspension of spores pipetted onto the sprayed surfaces. Preferably, 4 drops are placed on each slide and 4 slides in a standard moist chamber—a large Petri dish containing a glass slide rack and assembled, cover down, to permit the use of a water seal. The sealed chambers are set aside for 12 to 18 hours—or more—at desired temperatures to allow time for germination. Counts of germinated and ungerminated spores are made either under the low or high power of the microscope.

The procedure outlined has the virtue of simplicity, and, with minor modifications, has proved its worth in the hands of numerous investigators. In the writer's experience with it, however, the method is faulty in that it makes no provision for standardization and stabilization of the test drops on sprayed slides. If the spray contains a wetting agent the drops containing the spores tend to flatten and spread, frequently flowing together or off the slide during their 12 to 18 hours in the moist chamber. If the dried spray residue is water-repellent the drops tend to round up as flattened spheres. A slight jar in handling, however, may cause them to run together or off the slide. Naturally, the loss of drops lowers the efficiency of the test. Lack of uniformity in depth and configuration of the drops also lowers efficiency and may affect spore germination.

It is the purpose of this paper to outline a procedure whereby practical standardization and stabilization of test drops can be attained. The procedure was applied by the writer to extensive tests with sulphur fungicides at the University of Delaware in 1933 and 1934. It is particularly well-adapted to the evaluation of fungicides containing wetting and spreading agents, but can be used also to evaluate other sprays.

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[1] Approved by the Director of the Delaware Agricultural Experiment Station Feb. 8, 1941.

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MODIFIED SPORE-GERMINATION TECHNIQUE

The writer's method is similar in principle to that described in 1935 by Montgomery and Moore (6), who pipetted measured amounts of test materials onto 15-mm. circles cut into glass slides. In the writer's procedure 12-mm. glass circles, such as are in common use as cover glasses, are glued to the slides with petrolatum where they serve as standard surfaces onto which measured amounts of nutrients, toxic solutions and spore suspensions are pipetted either separately or in combination. In setting up a test the glass circles are washed in soap solution, boiled in potassium dichromate-sulphuric acid cleaning solution, and stored in distilled water. Just prior to use they are boiled in distilled water, transferred to paper toweling to dry, picked up singly with cover-glass forceps, flamed quickly, and deposited while still hot about 1 mm. apart on 75 × 25 mm. glass slides, the upper surfaces of which have been coated with a thin smear of petrolatum. Five circles are placed along each side of a slide, final spacing being effected by pressing lightly with cover-glass forceps on the center of each circle, and, with slight oscillating movement, forcing it into position. Excess petrolatum and air bubbles are squeezed from under the circles with the same movement, the petrolatum forming a low ridge around each circle, which acts as an effective barrier against bridging between drops.

The technique of coating the slides with petrolatum is important. Only enough—less than the thickness of a glass circle—is smeared on each slide with the finger to permit squeezing any air bubbles from under the circles. If used in excess, the petrolatum will tend to creep over the edges of the circles and encroach on the surfaces onto which the test materials are to be applied.

Toxic solutions and spore suspensions may be pipetted, separately or in combination, in measured amounts onto the circles. In tests with sulphur fungicides, however, the writer found it expedient to transfer the fungicide to the circles with a solid glass rod, about 3/16 in. in diameter and 6 in. long, one end of which had been polished crosswise on a fine carborundum cloth. In setting up a test, a 10-ml. quantity of the aqueous sulphur suspension was placed in a 100-ml. beaker, swirled and tilted to stir, leveled, and a single drop picked up by dipping the polished end of the rod into the suspension the full depth of the liquid—about $\frac{1}{4}$ inch. The rod was removed vertically with a quick movement and the resulting hanging drop was applied to the center of a circle and teased to the margin by a slight oscillating and vertical movement of the rod. A single drop was applied to each circle; rates of application were varied by diluting the original suspension and proceeding with 10-ml. quantities, as before. The aqueous spore suspension was applied in sufficient amount—usually two drops—to completely flood the circle (Fig. 1).

The advantages of this procedure are readily apparent. The capacity of each slide is more than doubled—10 test drops are used in place of the usual 4. Fungicidal dosage is accurately controlled and variability in depth and configuration of drops is practically eliminated. The tendency of drops to bridge and flow together or off the slide is almost entirely overcome. Slides can be jarred without loss of drops. Handling of slides while assembling the test and in making germination counts, therefore, is greatly facilitated.

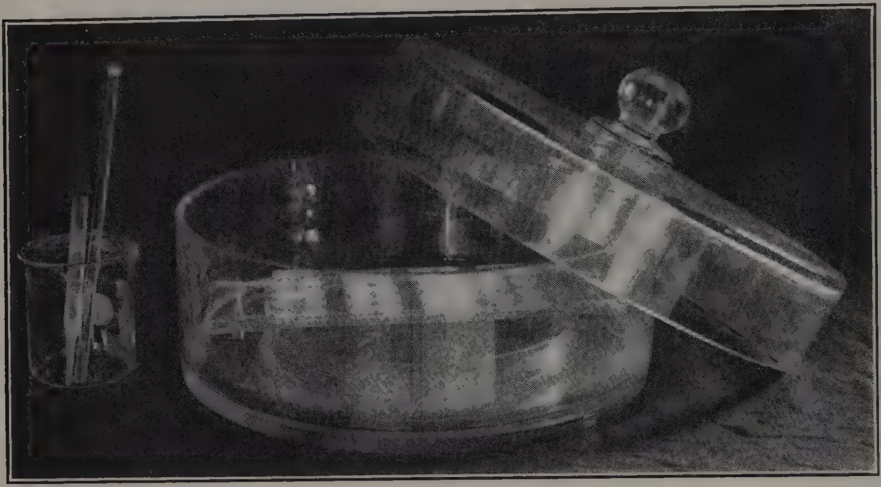

FIG. 1. Moist chamber containing improvised slide rack holding 4 slides in spore-germination test. Each 75 × 25 mm. slide carries 10 test drops on chemically clean 12-mm. glass circles glued to slide with petrolatum. Lower end of glass rod is used to pick up hanging drop of sulphur suspension in water for transfer to circle. Pipette is used for adding spore suspension in nutrient solution. Beaker (100 ml.) serves as container for sulphur suspension.

EXPERIMENTAL RESULTS

No attempt was made to establish the superiority of the modified procedure over that used by McCallan and others. It was readily apparent that the standardization of test drops greatly reduced variability in spore germination within a test. Germination continued to vary from test to test, however, dropping to 30 per cent in some tests and approximating 100 per cent in others. A quick study of factors affecting germination of *Sclerotinia fructicola* revealed that the manner in which the conidia were removed from culture greatly influenced germination. If removed with needle or brush, so as to avoid contact with the substratum, they germinated weakly or not at all. If washed quickly from culture with distilled water, germination was low and weak; if removed from test-tube culture on potato-dextrose agar, only after prolonged shaking with distilled water, germination was high and vigorous. Since this indicated the need of nutrients to insure satisfactory germination studies were made to determine suitable kinds and amounts.

Data relating to the effect of spore concentration and nutrients on spore germination are given in table 1. Comparisons are made between spores removed from test-tube culture on potato-dextrose agar with a camel-hair brush, and spores washed from the same culture with distilled water. Spore concentrations were determined by counting the spores at the greatest spore dilution in 20 microscopic fields 380 μ in diameter.

TABLE 1.—*Effect of spore concentration and nutrients on the germination of conidia of Sclerotinia fructicola grown on potato-dextrose agar*

Spores removed from culture with camel-hair brush						Spores washed from culture	
	Percentage germination in						
Spores per high-power field	Wash water from fresh p–d agar	Wash water from old p–d agar	Wash water from culture on p–d agar	0.01 per cent dextrose	Distilled water	Spores per high-power field	Germination in distilled water
							Per cent
112	100	94	13	31	9	120.0	98
56	100	99	61	53	10	60.0	99
28	100	99	91	40	5	30.0	100
14	100	100	95	28	1	15.0	99
7	100	100	95	11	0	7.5	99

Referring to table 1, it is quite apparent that sufficient nutrients were extracted in the process of washing spores from culture on potato-dextrose agar with distilled water to insure high and uniform germination—98 to 100 per cent—at all spore dilutions. Spores removed from the same culture with a camel-hair brush so as to avoid nutrient contamination germinated weakly or not at all in distilled water. Dilute dextrose solution was slightly to moderately stimulatory, depending on spore concentration; extracts of potato-dextrose agar were moderately to highly stimulatory, depending on age and previous treatment of the agar. The results serve to explain the erratic germination of conidia of *Sclerotinia fructicola* removed from common substratum in variable manner, but are not sufficiently quantitative to provide a satisfactory basis of procedure.

Tables 2 and 3 represent experiments separated in point of time by 2 days. Both percentage germination and germ-tube length are given as indices of the effect of nutrients, spore concentration, and sulphur on the germination of conidia of *Sclerotinia*. Difco potato-dextrose agar and bacto-dextrose were used as the source of nutrients. The potato-dextrose agar extract was made by macerating 2.0 g. of the dry powder in 100 ml. of water for 1 hour with occasional shaking, filtering to remove solids, and autoclaving at 15 lb. steam pressure for 20 minutes. The sulphur, a microfine paste made up of particles 5 μ in diameter and finer, was diluted to a 0.002 per cent sulphur concentration for use in these tests. Spores were removed from 12- and 14-day-old cultures on potato-dextrose agar with a platinum needle. Nutrients, sulphur, and spores were pipetted separately onto the glass circles, resulting in a further dilution of the nutrients and sulphur to about $\frac{1}{5}$ the concentration given in the tables.

TABLE 2.—*Effect of potato-dextrose-agar extract, spore concentration, and sulphur on the germination of conidia of Sclerotinia fructicola. Germination period: 17½ hours*

Spores per high-power field	2.0 g. p–d agar		0.5 g. p–d agar		0.125 g. p–d agar		0.032 g. p–d agar		Distilled water	
	Germination	Germ-tube length	Germination	Germ-tube length	Germination	Germ-tube length	Germination	Germ-tube length	Germination	Germ-tube length
	Per cent	*Microns*	*Per cent*	*Microns*	*Per cent*	*Microns*	*Per cent*	*Microns*	*Per cent*	*Microns*
112.0	99	[a]	80	300	43	100	1	50	trace	5
56.0	100	[a]	97	350	87	150	9	75	trace	5
28.0	100	[a]	97	400	97	200	75	75	trace	5
14.0	100	800 +	99	500	99	250	74	75	0	......
7.0	99	800 +	100	600	97	300	69	75	0	......
3.5	100	800 +	99	800	99	350	84	100	0	......
Plus sulphur										
112.0	89	500 +	38	200	trace	50	0	....	0	......
56.0	91	500 +	56	350	15	150	trace	50	0	......
28.0	85	400	54	300	16	150	1	50	0	......
14.0	45	100	36	200	6	50	trace	5	0	......
7.0	47	100	14	100	1	50	0	....	0	......
3.5	40	75	11	75	0	....	0	....	0	......

[a] Impossible to measure accurately because of crowding of spores and profuse germ-tube growth.

TABLE 3.—*Effect of dextrose, spore concentration, and sulphur on the germination of conidia of Sclerotinia fructicola. Germination period: 23 hours*

Spores per high-power field	2% dextrose solution		0.5% dextrose solution		0.125% dextrose solution		0.032% dextrose solution		Distilled water	
	Germination	Germ-tube length	Germination	Germ-tube length	Germination	Germ-tube length	Germination	Germ-tube length	Germination	Germ-tube length
	Per cent	*Microns*	*Per cent*	*Microns*	*Per cent*	*Microns*	*Per cent*	*Microns*	*Per cent*	*Microns*
176.0	83	200	83	200	57	100	3	50	0	......
88.0	91	200	88	150	88	150	46	75	trace	5
44.0	95	150	93	150	91	100	69	75	0	......
22.0	91	100	86	75	82	75	75	75	0	......
11.0	83	75	84	75	83	75	65	50	0	......
5.5	18	25	2	20	2	25	1	25	0	......
Plus sulphur										
176.0	58	150	57	150	49	150	1	25	0	......
88.0	47	200	46	200	41	150	4	100	0	......
44.0	22	150	26	150	59	200	17	200	0	......
22.0	16	150	8	150	8	150	4	150	0	......
11.0	1	75	trace	75	trace	75	trace	100	0	......
5.5	trace	50	trace	50	trace	50	trace	50	0	......

The experiments are reviewed together to compare trends set up by the several variables. Dextrose solution was less stimulatory than potato-dextrose agar extract. Germination decreased as nutrients decreased, whether the nutrient was dextrose or potato-dextrose agar extract. In potato-dextrose agar extract at minimal nutrient levels, germination increased as spore concentration decreased. In dextrose solution germination tended to reach a maximum at the intermediate spore concentrations, decreasing toward either extreme.

The effective dosage range of potato-dextrose agar extract is indicated in table 2. The extract made from 2.0 g. Difco potato-dextrose agar was fully stimulatory at all spore concentrations; the 0.5 g. extract induced high germination at and below 56 spores per high-power field; the 0.125 g. extract induced high germination at and below 28 spores per high-power field; the 0.032 g. extract failed to induce satisfactory germination at any spore concentration.

The inclusion of sulphur in duplicate experiments reduced percentage germination in dextrose solution and potato-dextrose agar extract, reduced germ-tube growth in potato-dextrose agar extract, and increased germ-tube growth in dextrose solution (Table 3). The growth-stimulatory effect of sulphur in dextrose solution can best be ascribed to the nutrient nature of Bindex, the wetting agent used in processing the sulphur. Supplementary tests with Bindex, a waste sulphite-liquor concentrate, proved it to be stimulatory.

DISCUSSION

Data listed in tables 1, 2, and 3 supplement information published by McCallan and Wilcoxon (4, 5) and Wilcoxon and McCallan (8) regarding the stimulatory effect of aqueous plant extracts on fungous spores. In general, the data support the 1939 findings (5) of these authors relative to *Sclerotinia fructicola.* None of the several isolates of *Sclerotinia fructicola* from peach used by the writer in studies with sulphur fungicides produced conidia that would germinate satisfactorily in distilled water. These findings add weight to accumulating evidence that the high germinability of the conidia of this fungus in distilled water, as reported in the literature, probably was due to nutrient contamination. The common procedure of washing spores from culture, whether decayed fruit or artificial media, will usually supply sufficient nutrients to stimulate high germination, although it is frequently necessary to add nutrients to induce vigorous germination.

As to sources of nutrients, Wilcoxon and McCallan (8) have called attention to the stimulatory properties of tomato, orange, apple, or pear juice, and aqueous extract of lily bulbs and gladiolus corms, and dahlia or potato tubers. The writer found Difco potato-dextrose agar a satisfactory and convenient material with which to make nutrient extracts. Extract of prune agar proved somewhat less stimulatory. Dextrose alone was weakly to moderately stimulatory.

Dilution of spores produced different effects in different nutrients. In potato-dextrose agar extract at low nutrient levels, germination increased when spore concentration was decreased; in dextrose solution germination was high at intermediate spore concentrations and low at either extreme. At the intermediate concentrations in dextrose solution the spores appeared to be mutually stimulatory, possibly because of substances from within the spores or of trace nutrients carried on the surface of the spores. Spore washing and centrifuging tests by McCallan and Wilcoxon (4) and perfusion tests by Goldsworthy and Green (1) indicate the probability of surface contamination. However, the failure of dextrose to stimulate germination at extremely low spore concentrations may have been because of trace amounts of toxic substances in the test materials, the effect of which was masked at the higher spore concentrations and when the more stimulatory potato-dextrose agar extract was used. This assumption is borne out by the fact that when sulphur was added to the potato-dextrose-agar extract (Table 2) the resulting germination trends closely approximated those of dextrose without sulphur (Table 3).

Recent spore-germination studies by Lin (2) bear on this same point. Lin finds that when dextrose is used as the sole stimulatory agent for conidia of *Sclerotinia fructicola*, germination is affected adversely by trace impurities in the glassware and distilled water. The test, in fact, is so sensitive that it would seem to preclude the use of dextrose as the sole stimulant in routine tests of fungicides. The more highly stimulatory substances, such as orange juice or potato-dextrose-agar extract, would seem to be the better choice.

The depression of germination due to crowding of spores at high spore concentrations (Tables 2 and 3) can be overcome by increasing the amount of nutrient. This, however, is of no practical significance in fungicidal tests, since, at these spore concentrations, the tendency of the spores to clump together makes germination counts difficult and inaccurate. In the writer's experience, about 20 spores per high-power field of 380 μ diameter is a desirable maximum for conidia of *Sclerotinia*. At this spore concentration, a nutrient solution made by extracting 0.1 g. of Difco potato-dextrose-agar powder in 100 ml. of distilled water for 1 hour usually will provide satisfactory germination. To decrease the sensitivity of the test the nutrient concentration should be increased; to increase sensitivity the nutrient concentration or spore concentration or both should be decreased.

Prior to routine use in spore germination tests it is suggested that each lot of potato-dextrose agar be subjected to biologic assay. The type experiment represented by the upper half of table 2 should be useful for this purpose.

SUMMARY

The common procedure of germinating fungous spores in drops of water on glass slides that have been sprayed with fungicides, as in the spore-

germination method of testing fungicides, makes no provision for the standardization and stabilization of the drops on the slides.

A modified procedure is outlined whereby practical standardization and stabilization of drops can be attained by gluing 12-mm. glass circles to the slides with petrolatum, where they serve as standard surfaces onto which measured amounts of fungicides and spores are pipetted either separately or in combination. Through this procedure a single 75 × 25 mm. slide can be made to carry as many as 10 different treatments. A hanging-drop method of applying the fungicide is described.

Conidia of *Sclerotinia fructicola* from peach failed to germinate satisfactorily in distilled water when so removed from sporulating surface as to avoid contamination with nutrients from the culture media. Of the several nutrients tested, including dextrose, prune-agar extract, and potato-dextrose-agar extract, the latter proved to be the most highly stimulatory to the conidia of *Sclerotinia fructicola.* A nutrient solution made by extracting 0.1 g. of Difco potato-dextrose agar powder with 100 ml. of water was found to produce high percentage germination. An experiment is outlined whereby each lot of the dry powder can be assayed as to stimulatory dosage.

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LITERATURE CITED

1. GOLDSWORTHY, M. C., and E. L. GREEN. Effect of low concentrations of copper on germination and growth of conidia of *Sclerotinia fructicola* and *Glomerella cingulata.* Jour. Agr. Res. [U.S.] 56: 489–505. 1938.
2. LIN, C. K. Necessary precautions in germinating purified conidia of *Sclerotinia fructicola* (Wint.) Rehm. (Unpublished data submitted as a term paper in Plant Pathology Course No. 201, Cornell University.) 1940.
3. MCCALLAN, S. E. A. Studies on Fungicides. II. Testing protective fungicides in the laboratory. Cornell University Agr. Exp. Stat. Memoir 128: 8–24. 1930.
4. ——————, and F. WILCOXON. The action of fungous spores on Bordeaux mixture. Contrib. Boyce Thompson Inst. 8: 151–165. 1936.
5. ——————, and ——————. An analysis of factors causing variation in spore germination tests of fungicides. I. Methods of obtaining spores. Contrib. Boyce Thompson Inst. 11: 5–20. 1939.
6. MONTGOMERY, H. B. S., and M. H. MOORE. On a new method for precision testing in the laboratory of the toxicity of lime-sulphur and of Bordeaux mixture as protective fungicides. Ann. Rep. East Malling Res. Stat. 1934: 217–222. 1935.
7. REDDICK, D., and E. WALLACE. On a laboratory method of determining the fungicidal volume of a spray mixture or solution. Science 31: 798. 1910.
8. WILCOXON, F., and S. E. A. MCCALLAN. The stimulation of fungus spore germination by aqueous plant extracts. Phytopath. (Abstract) 24: 20. 1934.

CHANGES IN NITROGEN AND VIRUS CONTENT OF DETACHED TOBACCO LEAVES IN DARKNESS

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(Accepted for publication March 22, 1941)

INTRODUCTION

The utilization of proteins, as well as carbohydrates, in the respiration of starved leaves has been shown by early workers through the use of detached leaves. When leaves detached from plants are cultured in distilled water a gradual increase in water-soluble nitrogenous substances arising largely from the steady hydrolysis of protein has been shown to take place. A conspicuous decrease in protein nitrogen in detached tobacco leaves was recently shown by Vickery, Pucher, Wakeman, and Leavenworth (7) to occur when cultured in distilled water, dilute glucose solution, or nutrient mineral solution, regardless of whether they were stored in light or in darkness. However, after the first 72 hours the rate of protein disintegration diminished where opportunity for photosynthesis was afforded. As would be expected leaves cultured in distilled water in the dark showed the greatest protein hydrolysis.

The culture of detached leaves in water may be considered as a type of starvation, where katabolic processes continue and synthesis is retarded or even halted, leading to premature degeneration of leaf tissue. A study of the changes in nitrogen and virus concentration in such leaves was undertaken with the hope that some knowledge as to the relation of host metabolism to tobacco-mosaic virus increase might be gained.

EXPERIMENTAL WORK

Changes in Nitrogen During Dark Culture

The changes in the distribution of nitrogen and in virus concentration were studied in 3 series of detached tobacco leaves, as follows: (1) *Mosaic leaves.* Leaves taken from plants inoculated 4 weeks previous to detachment, (2) *Mosaic-inoculated leaves.* Leaves inoculated immediately after detachment, (3) *Healthy leaves.* Noninoculated leaves from healthy plants.

In each series 4 comparable lots of 20 mature leaves each were taken from 20 uniform Turkish tobacco plants. One lot from each series (0 day) was shredded, placed in a covered glass container, and frozen immediately on harvesting, while the remaining 3 were placed with petioles in distilled water and stored in a darkened room at about 25° C. After the lapse of 3, 6, and 9 days a lot was withdrawn from each series, shredded, and frozen until ready for analysis.

Procedure for Following Changes in Nitrogen.—For analysis the frozen lot was passed through a meat grinder, weighed into 2 equal subsamples, and, by adding M/10 Na_2HPO_4, each was brought to a weight twice that of the original fresh weight of the subsample.

One subsample was used for the study of nitrogen changes and the other to determine virus concentration. The juice was extracted from each subsample by wringing the diluted macerated tissue in cheesecloth. The pulp was extracted twice with enough distilled water so that the weight of the pulp plus that of the extract would be 4 times the original fresh weight of the subsample. The pulp, together with the deposit resulting from the centrifugation of the extract, was dried for 10 hours in an oven at 60° C. This mixture is referred to hereafter as the leaf residue.

The distribution of nitrogen and the extent to which proteins were hydrolysed during the culture were followed by separating the nonhydrolysed protein from the hydrolysed portion in the extract with trichloracetic acid. The precipitate that is formed when this reagent is added has been considered to contain all nonhydrolysed native and virus protein; and the supernatant liquid, the products of hydrolysis. The precipitate was readily removed by centrifugation. The clear supernatant liquid, the precipitate,

TABLE 1.—*The changes in kjeldahl nitrogen in detached tobacco leaves during dark culture.* (*Figures denote milligrams of nitrogen per 100 grams of original fresh weight.*)

Series	Fraction		Duration of dark culture			
			0 day	3 days	6 days	9 days
Mosaic	Supernatant liquid	Exp. 1	67.4	93.3	154.5	163.4
		2	51.1	84.0	100.5	97.2
		3	39.3	75.6	105.7	107.9
	Trichloracetic precipitate	Exp. 1	98.0	91.5	88.5	83.2
		2	65.9	61.5	60.6	59.2
		3	75.9	93.7	85.8	82.6
	Leaf residue	Exp. 1	287.6	210.0	219.6	198.0
		2	197.2	154.0	136.4	147.2
		3				
Mosaic-inoculated	Supernatant liquid	Exp. 1	45.3	91.2	110.6	105.2
		2	47.3	75.5	107.3	112.7
		3	42.1	61.9	83.5	99.8
	Trichloracetic precipitate	Exp. 1	23.2	23.1	27.3	28.9
		2	32.1	29.7	27.0	25.5
		3	36.3	33.8	24.9	26.1
	Leaf residue	Exp. 1	135.8	87.6	78.6	64.8
		2	156.0	140.4	95.6	92.0
		3	148.8	125.6	73.6	88.0
Healthy	Supernatant liquid	Exp. 1	35.3	75.8	97.6	98.5
		2	39.7	66.2	101.6	106.4
		3	48.9	63.9	89.2	107.5
		4	39.5	64.9	84.5	91.1
	Trichloracetic precipitate	Exp. 1	26.5	23.8	23.7	20.9
		2	33.1	38.9	24.1	22.6
		3	36.2	31.9	26.9	26.1
		4	17.5	19.2	21.1	20.2
	Leaf residue	Exp. 1	135.8	89.2	71.8	65.6
		2	121.2	96.8	73.6	73.2
		3	122.0	112.4	90.8	84.0
		4	134.0	113.6	76.1	74.0

and the leaf residue were analysed for total nitrogen by the microkjeldahl method. The increase of nitrogen in the supernatant liquid or the decrease of nitrogen in the precipitate and leaf residue has been taken to indicate the degree of proteolysis.

Hydrolysis of Leaf Protein.—In all 3 series the hydrolysis of protein is quite noticeable. Table 1 shows that after 9 days there has been an increase of more than twofold in trichloracetic-soluble nitrogen (supernatant liquid). The concurrent decrease in leaf residue nitrogen suggests that much of the trichloracetic-soluble nitrogen was derived from this source. Furthermore, that this leaf-residue nitrogen was protein nitrogen was indicated by the work of Vickery and his associates (7) who extracted a fraction corresponding to our leaf residue with alkali and proved by various chemical tests that the extracted material consisted largely of proteins. When their extracted residue was treated with proteolytic enzymes a large part of the nitrogen was rendered soluble.

Although the nitrogen of the precipitate also tends to decrease, the differences are not very striking nor in all cases consistent.

In the tendency of their proteins to undergo hydrolysis during dark culture, the behavior of mosaic and mosaic-inoculated leaves was found not to differ essentially from that of the healthy leaves.

Changes in Virus Concentration During Dark Culture

In detached leaves from diseased plants (mosaic leaves) or in freshly inoculated leaves (mosaic-inoculated) water-cultured in the dark, the virus protein would be expected either to (a) be digested by the living plant cell during respiration as are most normal leaf proteins; (b) resist digestion but cease to increase; or (c) continue to increase in the face of increased proteolysis.

Biological Method of Determining Virus Concentration.—The increase or decrease of active virus during the progress of culture was followed by the usual biologic method of inoculating opposite halves of *Nicotiana glutinosa* leaves (2, 5) with extracts from leaves made at each succeeding interval. Since it was possible that normally unextractable virus might be released from the degenerating leaf tissue or might become more completely disaggregated, and thereby lead to an erroneous conclusion regarding virus concentration, extracts from subsamples that had been treated with trypsin to disaggregate the virus were used for inocula.

To one set of subsamples 2 mg. of Fairchild's trypsin was added to each gram of tissue based on its original fresh weight, adjusted to pH 7.5, preserved with toluene, and incubated for 36 hours at 38° C. After the juice had been extracted as described above, a portion of each subsample was heated for 20 minutes at 70° C. to inactivate the trypsin (1). It was found that inocula prepared in this way show an increased activity when compared with nontreated controls.

Resistance of Virus to Nitrogen Changes.—When the primary lesions of each set of 20 corresponding half leaves are compared in experiments 2 and 3 of the mosaic series of table 2, there appears to be a slight increase in virus concentration with time of storage, *e.g.*, the 3 day period shows an increase in number of local lesions compared with the 0-day, the 6-day period over the 3-day, etc. The inconsistent results of experiment 1 are believed to be due to some error, introduced during the handling of the material.

TABLE 2.—*The changes in virus content of detached tobacco leaves during dark culture. Local lesions on 20 half leaves of Nicotiana glutinosa*

Series		Dilution	0 day	3 days	3 days	6 days	6 days	9 days
Mosaic	Exp. 1	1: 100	904	556	438	491	497	488
	2	1: 400	87	138	139	209	158	196
	3	1: 100	822	976	857	973	946	1059
Mosaic-inoculated	Exp. 1	1: 4	1	27	34	252	348	253
	2	1: 4	1	4	1	101	192	721
	3	......	......	......	......	......	......	......

These infectivity studies show that the virus, at least, did not decrease during the culture period, and, therefore, was not used by the leaf cells. Although virus was present in abundance, the starving plant cell was not capable of hydrolysing this foreign protein to maintain its own respiration.

The multiplication of virus in detached, healthy, tobacco leaves inoculated with tobacco-mosaic virus in sand culture was studied by Purdy (4). Since she made no attempt to select mature leaves or exclude light and thereby arrest photosynthesis and further growth during 4 to 5 weeks of culture, many of her leaves developed adventitious roots and elongated from 0.6 to 14 per cent. Although visible leaf symptoms were not observed, the multiplication of virus was demonstrated by successful transfers of the virus to healthy tobacco plants and by the formation of inclusion bodies. Holmes (3) has reported the increase of virus in the absence of chlorophyll and light. He inoculated etiolated pieces of potato stem with tobacco-mosaic virus, and kept these in sterilized test tubes in the dark. That increase in virus took place was shown by the increasing numbers of local lesions when pieces of stem were withdrawn over a period of 16 days and used for inoculation of *Nicotiana glutinosa* leaves. The increase of tobacco-mosaic virus in whole tomato plants under similar conditions was also studied by Holmes.

Increase of Virus During Dark Culture.—The slight increase in virus concentration shown by the mosaic series in table 2 might be attributed to several causes: (a) actual multiplication of virus, (b) release of virus from degenerating host cells; (c) dispersion of virus aggregates or (d) to a combination of these causes. If the virus concentration of detached leaves initially low in virus were found to increase under the same cultural conditions the virus increase might then be considered to be actual. The mosaic-inoculated series (leaves inoculated at the time of detachment) served to clarify this problem.

The infectivity studies of the mosaic-inoculated series (Table 2) indicate a progressive increase of virus. Experiment 2 shows that considerable virus has formed in 9 days. There is little room for doubt in this case that actual virus increase took place. The virus, moreover, has increased concurrently with host-protein breakdown, where degeneration of leaf tissue rather than growth is taking place and where meristematic cells probably are lacking. If it can be assumed from the finding that soluble nitrogen continued to increase during culture in darkness that the further synthesis of normal proteins by the host cells had ceased or had fallen to a low rate, then it would appear that the increase of virus protein is not dependent solely on the ability of the host cells to synthesize normal proteins.

Effect of Leaf-Starvation on Inclusion Bodies

The fate of inclusion bodies in leaves of the mosaic series was followed by stripping the lower epidermis from the cultured leaves at different intervals and examining the size, shape and structure of the bodies in an attempt to gain evidence regarding their degeneration. Although striate material and hexagonal plate-like bodies were quite numerous, no conspicuous change in appearance was observed. These abnormal cell inclusions apparently may not be drawn upon by the host for sustenance. In epidermal strips taken from leaves of the mosaic-inoculated series inclusion bodies were not visible in 9 days. It is entirely possible that inclusion bodies are not formed until a much higher concentration of virus is reached in the cell.

Effect of Starvation on Stream Double Refraction

The property of the extracts to exhibit stream double refraction was not destroyed by dark culture; a fact which indicates that the elongated form of the virus particles was not destroyed by the action of hydrolytic enzymes of the plant. Stream double refraction was detected in extracts from leaves of the mosaic-inoculated series, which had been cultured for 6 to 9 days. This shows that the virus had reached a sufficiently high concentration within 6 days to produce this phenomenon. Extracts from healthy leaves that had been frozen and centrifuged did not show stream double refraction in any of the lots.[1]

SUMMARY

During dark culture the tendency of proteins to undergo hydrolysis in mosaic and mosaic-inoculated leaves did not differ from that of the proteins in the healthy leaves.

Tobacco-mosaic virus multiplies in mature detached tobacco leaves stored in the dark with petioles in distilled water and hence does not seem to require meristematic cells or actively growing tissue for its multiplication.

Tobacco-mosaic virus multiplies under conditions where hydrolysis of host proteins has been enhanced and host proteins are low.

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[1] Extracts from healthy leaves show stream double refraction only when fresh. Freezing followed by centrifugation eliminates from the extract the material responsible for stream double refraction in healthy leaves (6).

Under these conditions the living tobacco cell is not capable of utilizing tobacco-mosaic virus protein to support its respiration. It apparently does not possess means by which this foreign protein can be hydrolysed.

After macerated infected tissues are treated with trypsin the extract shows a greater infectivity than extracts from nontreated tissues. This increase in infectivity probably is due to the disaggregation of the virus.

Crystalline inclusion bodies may not function as reserve protein and are not drawn upon by the starving host tissues to support its respiration.

Stream double refraction exhibited by tobacco-mosaic virus suspensions is not destroyed by prolonged culture in detached leaves in the dark.

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LITERATURE CITED

1. CALDWELL, JOHN. The physiology of virus diseases in plants. IV. The nature of the virus agent of aucuba or yellow mosaic of tomato. Ann. Appl. Biol. **20**: 100–116. 1933.
2. HOLMES, FRANCIS O. Local lesions in tobacco mosaic. Bot. Gaz. **87**: 39–55. 1929.
3. ——————. Increase of tobacco-mosaic virus in the absence of chlorophyll and light. Phytopath. **24**: 1125–28. 1934.
4. PURDY, HELEN A. Multiplication of the virus of tobacco mosaic in detached leaves. Amer. Jour. Bot. **15**: 94–99. 1928.
5. SAMUEL, GEOFFREY and J. G. BALD. On the use of primary lesions in quantitative work with two plant viruses. Ann. Appl. Biol. **20**: 70–99. 1933.
6. TAKAHASHI, WILLIAM N., and T. E. RAWLINS. Stream double refraction exhibited by juice from both healthy and mosaic tobacco plants. Science **77**: 284. 1933.
7. VICKERY, HUBERT BRADFORD, GEORGE W. PUCHER, ALFRED J. WAKEMAN, and CHARLES S. LEAVENWORTH. Chemical investigations of the tobacco plant. VI. Chemical changes that occur in leaves during culture in light and in darkness. Connecticut Agr. Exp. Stat. Bull. 399. 1937.

RELATION OF CERATOSTOMELLA RADICICOLA TO RHIZOSIS OF THE DATE PALM[1]

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INTRODUCTION

Rhizosis, a potentially serious disease of date palms, has been known in the Coachella Valley of California since 1933. The sudden loss of large, vigorous palms has caused considerable apprehension among growers in certain districts. Evidence that rhizosis probably is caused by a species of *Ceratostomella* was presented in a preliminary paper.[2] This fungus has recently been described under the name *C. radicicola* Bliss.[3]

During the first 3 years of the investigation (1933–1936), typical symptoms of rhizosis were studied in 21 palms located in 10 different date gardens, and the *Chalaropsis* stage of *Ceratostomella radicicola* was isolated from 10 palms in 7 gardens. In 1940 there was another outbreak of the disease, which caused the loss of approximately 80 palms. Isolations were made from the roots of 14 affected palms, but *C. radicicola* was not detected in any case. Although there can be little doubt as to the pathogenicity of this fungus on date palm, the question arises whether there may not be other, yet unknown, causal factors.

The first visible sign of rhizosis is a reddish-brown discoloration of the pinnae. Within a few days the leaves begin to wilt and die, the disturbance commencing in the lower whorls and progressing upward. The fruit stalks wilt suddenly; the dates, if still in the early stages of development, drop from the strands. Before all the older leaves have wilted, the tightly folded young leaves at the center of the crown lose turgor and become whitish. Affected palms may die within 4 to 6 weeks.

Observations made from dissected palms indicate that the roots become necrotic and filled with fungi at an early stage in the disease. The leaves and trunk are apparently free from fungal attack until the top of the palm is badly wilted. Then the tissues surrounding the terminal bud become infested with bacteria and fly maggots, and decay is rapid.

Since observations indicated that rhizosis is essentially a disease of the roots, attention was directed toward the factors of root injury. Many different species of fungi were isolated from the roots, but *Ceratostomella radicicola* was the only one that, in experimental inoculations, showed marked pathogenicity on date palms. It is the purpose of this paper to describe and illustrate the pathological effects of this organism on naturally and artificially inoculated date palms.

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[1] Paper No. 432, University of California Citrus Experiment Station, Riverside, California.

[2] Bliss, Donald E. Rhizosis, a recently discovered disease of date palms. Date Growers' Inst. Ann. Rept. 13: 4–6. 1936.

[3] Bliss, Donald E. A new species of *Ceratostomella* on the date palm. Mycologia 33: 468–482. 1941.

PATHOLOGICAL EFFECTS OF CERATOSTOMELLA RADICICOLA

Natural Infection

The symptoms of disease associated with *Ceratostomella radicicola* were clearly shown in 2 date gardens. A summary of the observations made on the affected palms is given to indicate the nature of the disease in the field.

Specimens were collected in October, 1934, from 2 large Deglet Noor date palms in the Johnstone garden. The bases of recently wilted leaves were, for the most part, free from fungus invasion. About one third of the primary roots were completely dead; many of the other primary roots contained islands of brownish, necrotic tissue. In some of these partly necrotic roots the lesions centered about the bases of secondary roots. Affected tissues from both the cortex and the central cylinder of the roots were taken aseptically from 32 root segments. *Ceratostomella radicicola* appeared in 15 cultures; *Trichoderma lignorum* (Tode) Harz, in 13; *Fusarium* spp., in 4; and *Diplodia phoenicum* (Sacc.) Fawcett and Klotz, in 1 culture.

In the Clum garden, in October, 1935, attention was called to a Deglet Noor palm that was dying rapidly. About one third of the leaves had wilted, and the fruit had become shriveled and worthless. At that time the youngest leaves appeared normal, although the trunk had not elongated more than 6 inches during the preceding year. Preliminary tissue cultures were made from segments of 20 roots which contained brownish, necrotic tissues. Five of these cultures contained *Ceratostomella radicicola;* 5, *Fusarium* sp.; 6, *Rhizoctonia solani* Kühn; 1, *Rh. bataticola* (Taub.) Butler; and 1, *Rhizopus* sp. Various unidentified forms of bacteria and nematodes also appeared in these cultures.

This palm was dissected 2 months later. It was first dug out, with a large mass of roots attached. Crosscuts through the trunk—one just below the terminal bud and the other at the soil line—indicated that the tissue of the trunk, the terminal bud, and the leaf bases were alive and apparently free from fungus invasion. About 30 per cent of the roots that were cut 1 foot from the trunk were brown and dead. Closer examination of these affected roots (Fig. 1) showed that necrosis had originated some distance from the trunk and had advanced inward. At the time of dissection many roots were found in which necrosis had extended within 10 to 15 cm. of the trunk. Tissue cultures from 14 affected roots yielded *Ceratostomella radicicola* in 11 cases. Some of the cultures were pure for *C. radicicola,* while others contained such organisms as *Trichoderma lignorum, Fusarium* sp., *Verticillium* sp. (?), bacteria, nematodes, and mites. Sections of diseased roots revealed masses of macrospores of the *Chalaropsis* stage of *C. radicicola* in the xylem ducts (Fig. 2, A) to points 2 cm. in advance of general root necrosis. These sections also showed mycelium and spores (Fig. 4, C) of the fungus in fibrous sclerenchyma cells surrounding the vascular elements and in the cortical region.

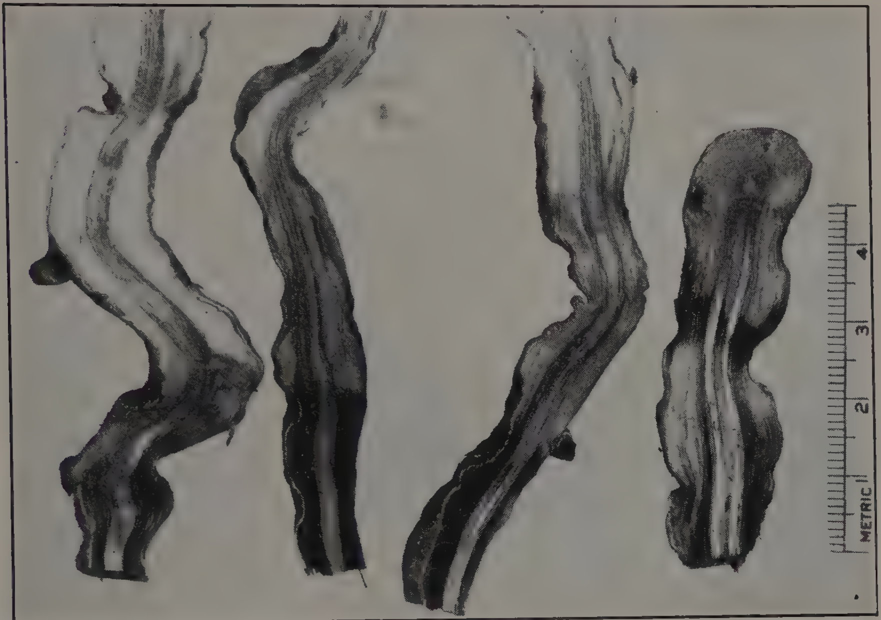

FIG. 1. Four root segments of a diseased Deglet Noor date palm. The roots were split longitudinally to show the dark, necrotic regions in the cortex and central cylinder, which had been invaded by *Ceratostomella radicicola.*

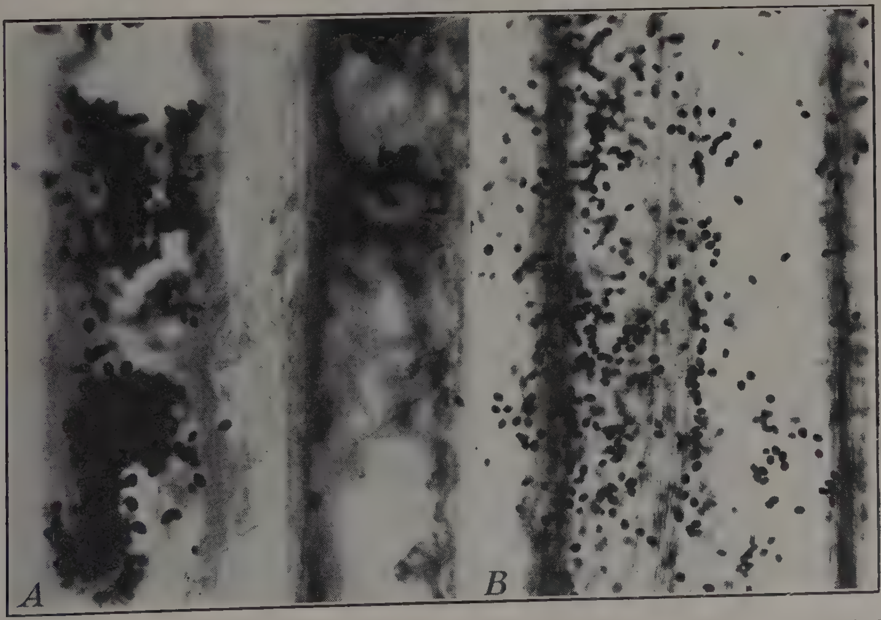

FIG. 2. Mycelium and spores of the *Chalaropsis* stage of *Ceratostomella radicicola* in the root of date palm. A. Masses of spores in the xylem ducts of a naturally infected palm (×116). B. Cortical tissue of an inoculated seedling palm, showing the destruction of the thin-walled parenchyma cells between the fibers (×113).

Artificial Inoculations

The first artificial inoculations were made by placing pure cultures of *Ceratostomella radicicola* (grown on bran) in the top soil about potted seedling date palms. These trials gave negative results. The next inoculations were made by inserting the organism (grown on potato dextrose agar) in wounds on leaf bases of larger seedling palms. These inoculations were wrapped with waxed paper and sealed with adhesive tape to prevent desiccation. The results were rapid and positive. Within 2 weeks the inoculated leaves wilted and died as a result of the rapid necrosis of the tissues surrounding the wound.

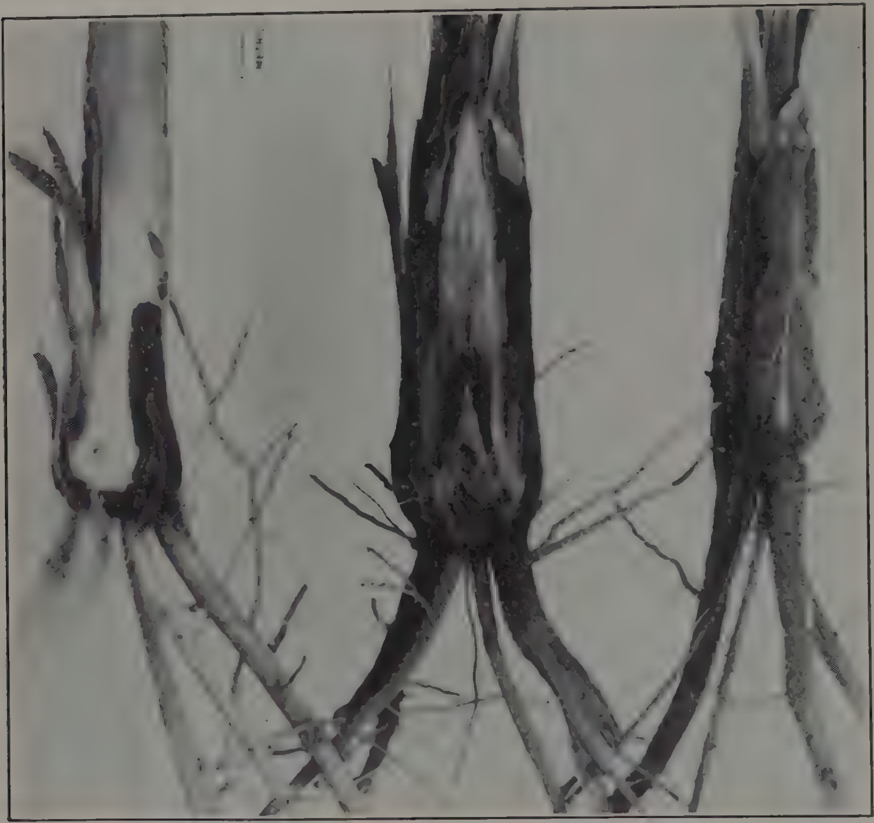

FIG. 3. Three 16-month-old wounded seedlings of Deglet Noor date palm 20 days after inoculation with *Ceratostomella radicicola:* left, control; center, plant cut longitudinally through the terminal bud to show darkening and necrosis of leaves and stem; right, another necrotic plant.

Finally, potted seedling-date palms were inoculated successfully by inserting moldy bran in wounds in leaf bases below the soil line. In one experiment, 16-month-old seedlings of Deglet Noor date palm were dead 20 days after inoculation through wounds (Fig. 3). Sections made through the affected plants revealed the mycelium and spores of *Ceratostomella* *radicicola* within the leaf bases, trunk, terminal bud, and roots. The leaf bases and primary roots were decayed and blackened by the fungus. The mycelium was found advancing through the parenchyma (Fig. 4, A) of the leaf bases, where macrospores were formed within the cells (Fig. 4, B). The parenchymatous tissues in the roots disintegrated, leaving blackened fibers and fungus spores in the region of the cortex (Fig. 2, B). The effect on the roots of the experimental plants was similar to that already described for naturally infected palms in the field.

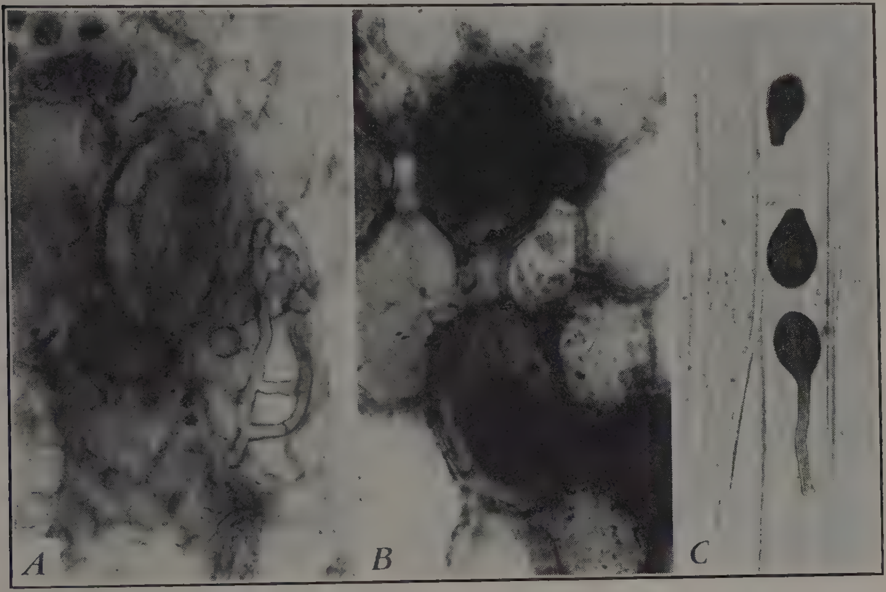

FIG. 4. Mycelium and spores of *Ceratostomella* within cells of the date palm. A. Cross section through the leaf base of an inoculated palm, showing branched mycelium in parenchyma cells containing starch grains (× 975). B. Macrospores formed within parenchyma cells of leaf base (× 975). C. Macrospores formed within a fibrous sclerenchyma cell of the central cylinder of a naturally infected root (× 445).

An experiment was undertaken to determine the effect of wounding on the susceptibility of seedling Deglet Noor palms to attack by *Ceratostomella*

TABLE 1.—*Relative susceptibility of wounded and nonwounded seedling Deglet Noor date palms to attack by Ceratostomella radicicola*

Culture	Plants nonwounded		Plants wounded	
	Number tested	Number infected	Number tested	Number infected
None (control)	7	0	8	0
B–252[a]	7	3	6	6
B–258[b]	6	0	6	5
B–252[a] and B–258[b]	7	4	4	4
B–312	7	3	7	7

[a] Single macrospore culture (plus strain).
[b] Single endospore culture (minus strain).

radicicola (Table 1). The fungus was grown on bran and placed about the bases of 16-month-old palms growing in soil in 5-gallon containers. Some of the plants were wounded below the soil line with a sterile scalpel. Three different cultures of the fungus were used, 2 of which represented the plus and minus strains of this heterothallic organism. The plants were examined after 4 months. The noninoculated controls were free from infection. While all but 1 of the wounded, inoculated plants were diseased, only 37 per cent of nonwounded, inoculated seedlings were affected. There was also a marked difference in the severity of attack on wounded and nonwounded plants. Where no artificial wounds had been made, the lesions were small, superficial, and centered about blemishes on the surface of the outer leaf bases. That these lesions were the result of inoculation was demonstrated by the reisolation of the fungus from necrotic tissues just below the epidermis. The different cultures were about equal in their pathogenicity.

Striking evidence of the action of *Ceratostomella radicicola* on tissues of the date palm was demonstrated in the laboratory. Nonwounded, green, Deglet Noor fruits were inoculated with a suspension of spores. On the 4th day after the inoculation, dates began to fall from the fruit strands and to undergo rapid decomposition (Fig. 5). Most of the dates in the control jar retained a normal appearance until the 11th day, when the experiment was ended. The ability of *Ceratostomella radicicola* to attack nonwounded green fruit is remarkable, since similar inoculations with *Aspergillus niger*

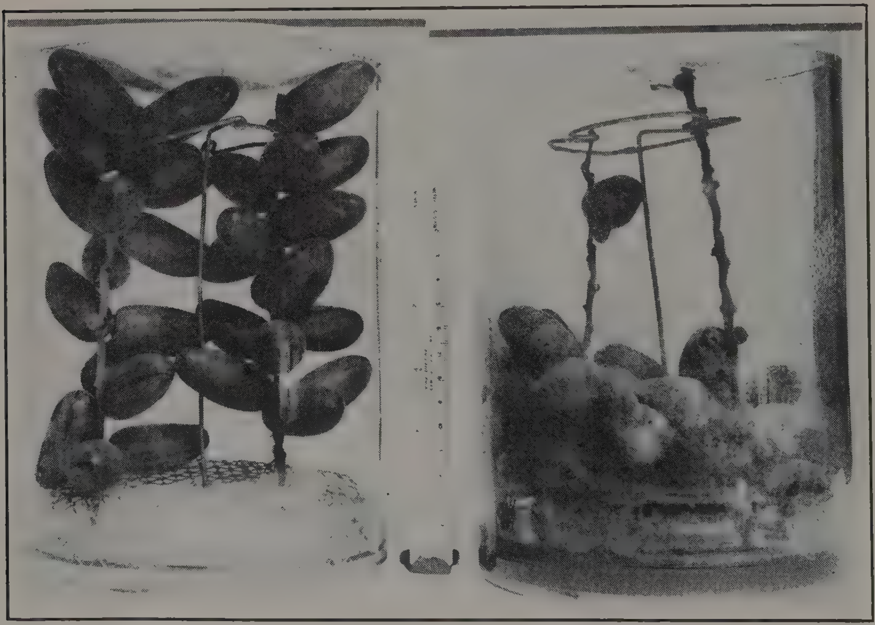

FIG. 5. Green nonwounded dates of Deglet Noor variety, 11 days after inoculation with *Ceratostomella radicicola:* left, control; right, inoculated fruit undergoing rapid decay.

van Tiegh. and *Alternaria citri* Pierce, two of the most important fruit-spoilage fungi, produced no effect.

CONCLUSIONS

Since *Ceratostomella radicicola* was found associated with rhizosis in less than one half of the cases investigated, there is reason to doubt whether this fungus is the only factor involved. In other words, it cannot be definitely stated that *C. radicicola* is the cause of rhizosis, for many mature palms die suddenly with all symptoms of rhizosis *except* the presence of *C. radicicola* in the roots. It may be reported with certainty, however, that this fungus is sometimes associated with rhizosis, and that, when inoculated into wounded seedling date palms, it causes rapid necrosis. Additional information regarding rhizosis must await further research.

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VERTICILLIUM WILT OF STRAWFLOWER[1]

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(Accepted for publication April 1, 1941)

INTRODUCTION

A serious wilt of strawflower (*Helichrysum bracteatum* Andr.), one of the most important of the everlasting flowers grown in the United States, was observed in plantings near Montara, San Mateo County, California, in the summer of 1938. Approximately ⅓ of the marketable crop of flowers was rendered unsalable just prior to picking, while, in 1939, ½, and in 1940, ⅔ of the crop was affected. Comparable losses also were sustained in field plantings on a coastal bench near Pescadero in the same county. This disease was found to be a limiting factor in all plantings, amounting to approximately 32 acres. In September, 1940, K. F. Baker reported to the writers its occurrence at Lompoc, Santa Barbara County, where the loss was less significant. Isolation and infection studies have shown that the disease is caused by *Verticillium albo-atrum* (Reinke and Berthold). A careful search of the literature indicates that strawflower has not previously been listed as a host of this fungus.

SYMPTOMS OF THE DISEASE

Strawflower wilt occurs only in field plantings, and has not been observed in the seedbeds. The first visible sign of infection usually appears at the commencement of the flowering period, or approximately 5 months after the seedlings are transplanted. The lower leaves of infected plants may wilt suddenly, without noticeable change in color. As the disease progresses, the upper whorls of leaves also wilt. Soon the wilted leaves become chlorotic; later, brown, necrotic areas, which later coalesce, develop along the margins (Fig. 1, B), in marked contrast to healthy leaves (Fig. 1, A). Still later, affected leaves become entirely dry and brown, remaining attached to the main stem of the plant but hanging in a vertical position. Although most of the leaves of infected plants show conspicuous wilting and browning in advanced stages of the disease, it is not uncommon to find a whorl of turgid leaves near or at the apex of the main stem. Internally, the stem shows a blackish-brown discoloration of the vascular tissues (Fig. 1, C), as compared with the healthy condition (Fig. 1, D), which may extend for a considerable distance above the soil level. Infection, if early, reduces the length of the stem internodes, with consequent stunting of the plants.

The external appearance of the roots is normal, but transverse or longitudinal sections show a discoloration of the vascular tissues similar to that in the stem. In both the roots and stems, apparently, the adjacent cortex remains healthy. Also, the structural stability of the stem and root tissues is not materially affected by fungus invasion, as indicated by the fact that infected plants, even after death, remain standing in the field. Diseased plants, however, are more easily pulled from the soil than healthy specimens.

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[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

[2] The assistance of nontechnical employees of the Federal Works Progress Administration is acknowledged; project 65-1-08-91.

Occasionally, infected plants die just prior to the flowering period, or die without producing flower buds. Generally, however, they develop flowers and may remain alive for a considerable time after infection.

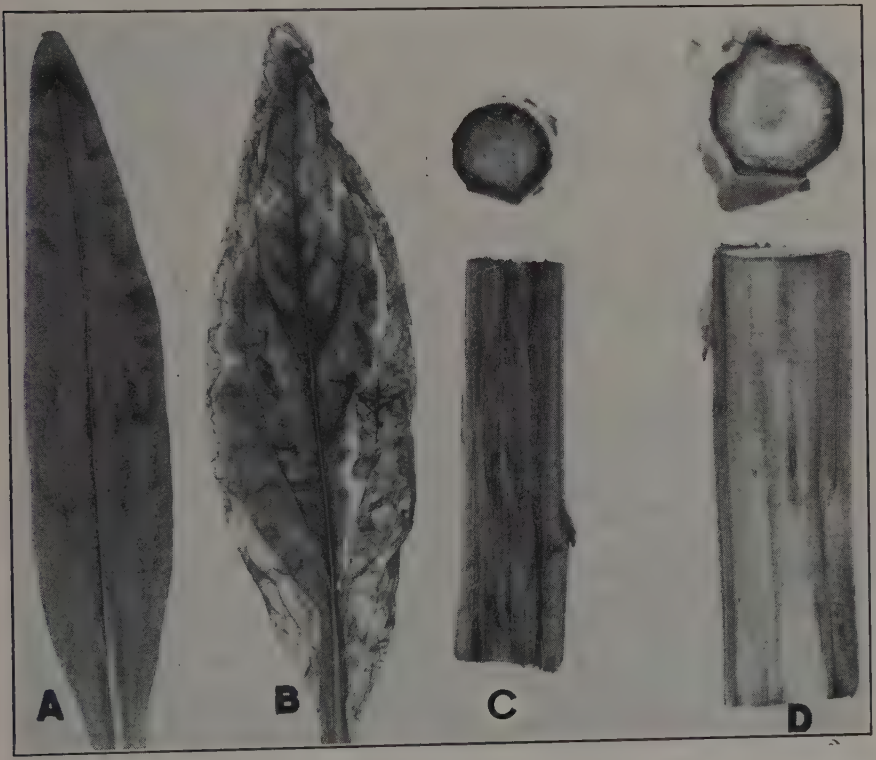

FIG. 1. Symptoms produced by *Verticillium albo-atrum* on strawflower. A. Healthy leaf. B. General chlorosis and marginal necrosis of a leaf from an infected plant. C. Cross and longitudinal sections of a diseased stem, showing vascular discoloration. D. Sections of a healthy stem.

Infected plants do not occur in localized areas but are scattered throughout the field. From a distance, diseased fields give the appearance of having been scorched by fire.

The principal effect of the disease, as far as the growers are concerned, is the reduction in number of high-grade flowers available for processing and marketing. Flowers produced on wilted plants are inferior in quality and size, and those that can be used yield only half as much revenue as the No. 1 grade flowers (having a diameter of $1\frac{1}{2}$ inches with a closed center after drying by artificial heat for 24 to 36 hours at 120° to 180° F.). Many flowers from diseased plants are so inferior in size and quality that they cannot be processed. In fields where the incidence of infection is high (most fields averaging 90 to 100 per cent), less than 5 per cent of No. 1 grade flowers are produced.

The cool, damp weather required for successful strawflower production is, likewise, ideal for the development and spread of the disease. Strawflower wilt has been found on soil types ranging from peat 30 feet deep to sandy, gravelly soils.

The Causal Fungus

The causal fungus, *Verticillium albo-atrum,* was readily isolated from the roots and stems of recently infected strawflower plants by placing small tissue fragments from the internal, advancing margin of decay on potato-dextrose and Czapek's agar in Petri dishes. After 4 to 6 days, pure cultures were established on agar slants by mass transfers from Petri-dish colonies. The fungus was cultured at intervals from approximately 100 diseased strawflower plants. Single-spore cultures of some of these isolates readily segregated into two classes, *i.e.,* conidial and mycelial types (Fig. 2, A and B), referred to by Hansen[3] as the dual phenomenon. These two types have been maintained unchanged through successive transfers for many months and appear to be highly stable.

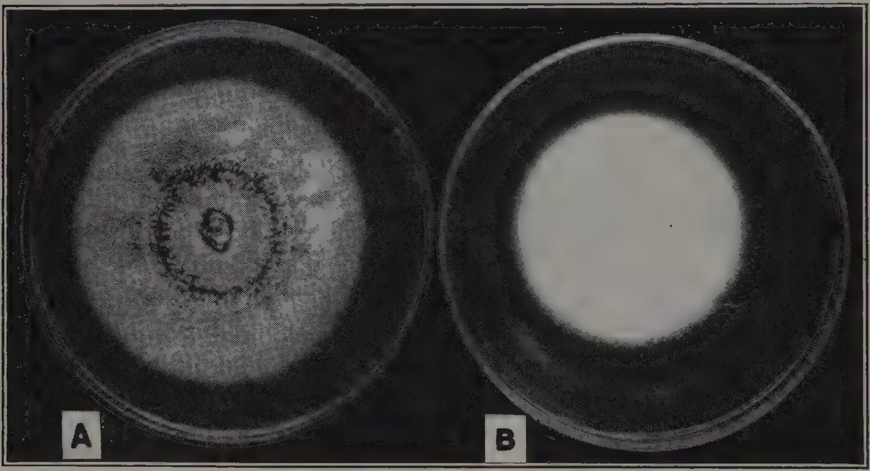

FIG. 2. Single-spore cultures of *Verticillium albo-atrum* from strawflower, 16 days old, on Czapek's agar. A. Conidial type. B. Mycelial type.

Results of studies on the morphology of the isolates from strawflower are in close agreement with the original description of *Verticillium albo-atrum* as given by Reinke and Berthold.[4] The identity of the fungus was verified also by H. N. Hansen of this Division.

The pathogenicity of the conidial and mycelial types of the fungus from strawflower was tested in the glasshouse. Inoculum, consisting of plate cultures of the fungus on Czapek's agar, was added in uniform quantity to autoclaved soil in 6-in. pots, each containing a young strawflower plant (6 to

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[3] Hansen, H. N. The dual phenomenon in imperfect fungi. Mycologia 30: 442–455. 1938.

[4] Reinke, J., and G. Berthold. Die Zersetzung der Kartoffel durch Pilze. Unters. Bot. Lab. Univ. Göttingen 1: 1–100. 1879. (Wiegandt, Hempel, und Parey. Berlin.)

8 in. high), in a manner designed to avoid wounding the roots. Sterile Czapek's agar was used for the noninoculated controls. Twenty plants each were inoculated with the conidial and mycelial types of the fungus, with an equal number for controls. The plants were grown at temperatures favorable to infection (55 to 65° F.) and were watered moderately. Typical symptoms developed on inoculated plants in each series in from 4 to 5 weeks and were identical with those encountered in the field (Fig. 3, B and C). At the end of the fifth week, all inoculated plants were infected, while the controls remained healthy (Fig. 3, A). The two types of the fungus which were reisolated proved identical with the original cultures. The reisolates of the two types again proved pathogenic in parallel inoculation tests. No difference in virulence between the conidial and mycelial types could be detected as a result of these tests; both were highly pathogenic.

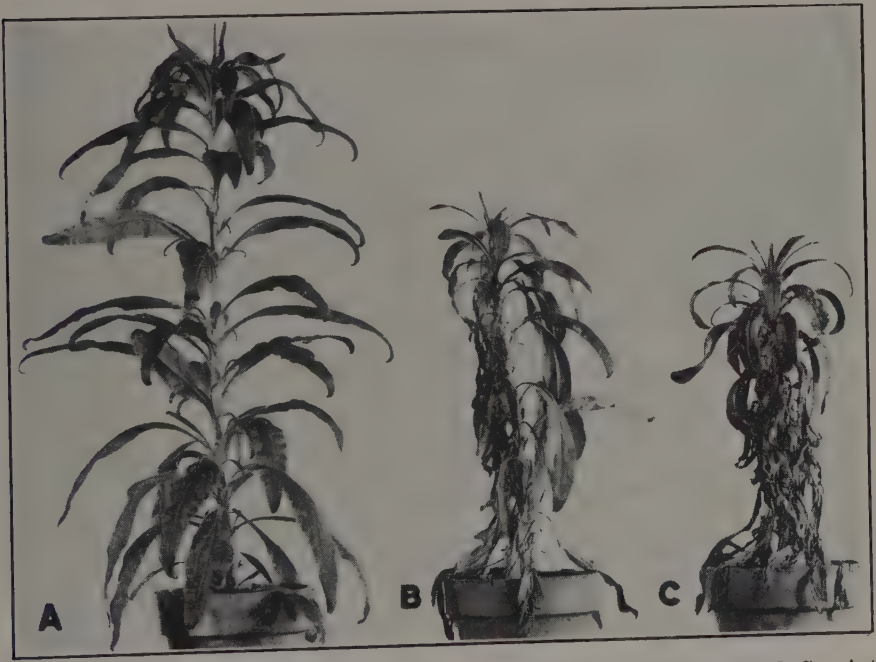

FIG. 3. Verticillium wilt of strawflower. A. Healthy control. B. and C. Artificial infection after 5 weeks in the glasshouse, showing typical wilting symptoms.

Tests were conducted in the field and glasshouse for evidence of seed transmission of the fungus. Strawflower seeds from naturally infected strawflower plants were treated in a 1–1,000 solution of mercuric chloride for 10 minutes, washed in tap water, dried, and then planted in flats of autoclaved soil in the glasshouse. Nontreated seeds also were planted for controls. From the treated lot of seed, 285 seedlings and from the nontreated lot 313 seedlings developed, all of which were free from infection. Approximately 100 seedlings from each lot were then transferred to 6-inch pots of autoclaved soil in the glasshouse and an equal number transplanted in the field at Berkeley, both lots being grown to maturity. All plants continued healthy through the flowering period, and the results indicate that the fungus probably is not seed-borne.

Limited inoculation tests were conducted in the glasshouse to determine the experimental host range of the strawflower isolates of *Verticillium albo-atrum.* Infection was obtained on Acala cotton (*Gossypium hirsutum* L.), eggplant (*Solanum melongena* L.), tomato (*Lycopersicum esculentum* Mill. var. *commune* Bailey), and sunflower (*Helianthus annuus* L.). The following plants were not infected: carnation (*Dianthus caryophyllus* L.), poppy anemone (*Anemone coronaria* L.), horseradish (*Armoracia rusticana* Gaertn.), *Geum chiloense* Balb., peach (*Prunus persica* Sieb. and Zucc.), potato (*S. tuberosum* L.), Turkish tobacco (*Nicotiana tabacum* L.), snapdragon (*Antirrhinum majus* L.), cucumber (*Cucumis sativus* L.), Canterbury bells (*Campanula medium* L.), Romaine lettuce (*Lactuca sativa* L. var. *longifolia* Lam.), China aster (*Callistephus chinensis* Nees), English daisy (*Bellis perennis* L.), cineraria (*Senecio cruentus* DC.), and *Helipterum roseum* Benth.

CONTROL

The verticillium wilt appears to be the chief limiting factor in the successful culture of strawflowers in California, other diseases, such as aster yellows and mildews, being of minor importance. During the past decade, it has annually been customary for growers to use the same land for transplanting of the new crop of strawflower seedlings. Since the causal organism, *Verticillium albo-atrum,* is a soil inhabitant and may persist in the soil for many years, observations showed that the disease has increased rapidly due to lack of proper rotation. Seed transmission of the organism has not been demonstrated and, therefore, control may be effected by using clean ground. In 1940, a small field of strawflowers at Montara, which had previously not been planted with this crop, was free from the disease.

SUMMARY

A serious vascular wilt of strawflower plants, with its attendant loss in salable flowers, occurs on the coast of central California.

The causal organism was identified as *Verticillium albo-atrum,* a common soil inhabitant. It readily segregated, upon single-sporing, into conidial and mycelial types.

Young strawflower plants were readily infected in 4 to 5 weeks under glasshouse conditions.

The fungus apparently is not seed-borne.

The strawflower isolates of the fungus were pathogenic to Acala cotton, eggplant, tomato, and sunflower.

The disease can be controlled by using clean soil.

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SALTANTS FROM A MONOSPORIC CULTURE OF VERTICILLIUM ALBO-ATRUM

JOHN T. PRESLEY[1]

(Accepted for publication April 10, 1941)

The occurrence of saltants in fungi is by no means a new or unusual phenomenon. Many investigators have reported on these relatively permanent changes in the appearance of fungi, and have in many instances shown differential pathogenicity between the parent and the saltant. It is the purpose of this paper to call attention to the frequent occurrence of saltants in a monosporic culture of *Verticillium albo-atrum* Reinke and Berthold, and its possible bearing on the taxonomy of the so-called species of this genus.

The parent culture of *Verticillium* used in this study was obtained by isolating single spores from a stock culture isolated in 1938 from a diseased chrysanthemum plant in a greenhouse near University Farm, St. Paul, Minnesota, and identified as *V. albo-atrum.* When grown on potato-dextrose agar in Petri plates occasional sectors were noted and by selective picking of these sectors cultures were obtained that differed widely among themselves (Fig. 1). In an effort to induce more rapid sectoring of the fungus various media were tried. The medium on which sectors appeared to be produced in greater abundance was of the following formula:

$MgSO_4$	0.5	g.	Asparagine	1.5	g.
KH_2PO_4	1.5	"	Dextrose	20.0	"
NH_4NO_3	0.05	"	Agar	20.0	"
KNO_3	2.0	"	Distilled water	1000	cc.

Each culture sectored freely, and a large number of cultural types was obtained, ranging from black to pure white and from very fluffy to completely submerged (Fig. 2). The production of microsclerotia in the different cultures was also variable. In some cultures there were only blackened septate hyphae; in others there were scattered, small microsclerotia; and in the black culture the microsclerotia were relatively large and very numerous, giving the entire surface of the culture a gritty appearance. With such latitude in the ultimate appearance of an isolate of *Verticillium* it is highly probable that errors unwittingly have been made in assigning the specific name to a given culture of the fungus.

To fully appreciate the importance of saltants to the taxonomic problems that have arisen, it may be desirable to recall some of the controversies that have developed. The genus was created in 1816 by Nees von Esenbeck (10). Corda (4) erected the genus *Acrostalagmus* in 1838 to accommodate a fungus *A. cinnabarinus,* which was said to differ from *Verticillium* by forming its spores in heads at the tips of the conidiophore. Hoffman (5), however,

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[1] The writer wishes to express his gratitude to Dr. E. C. Stakman for his advice and encouragement throughout the investigation and to other members of the Division of Plant Pathology, University of Minnesota, for assistance in various ways.

Paper No. 1899, Scientific Journal Series, Minnesota Experiment Station.

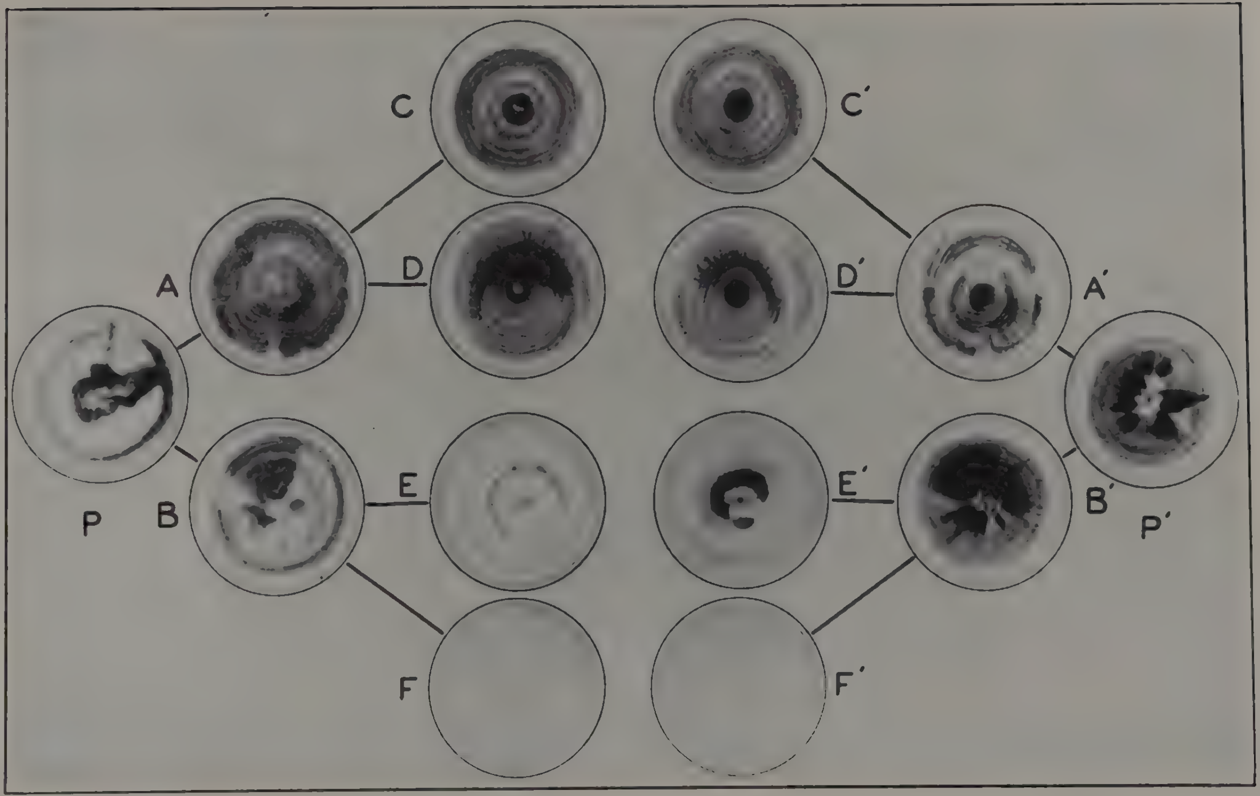

FIG. 1. Cultural types of *Verticillium albo-atrum* obtained by selective picking of sectors from a monosporic culture. The parent culture (P) of *V. albo-atrum* with 6 of the saltants (A–F) isolated from it. Note the range from completely microsclerotial (C) to completely mycelial (F) and the culture (E), which appears mycelial from above but shows microsclerotia when viewed from below. P′, A′, B′, etc., are the lower surfaces of P, A, B, etc. (Assistance in the preparation (drawings) of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 65–1–71–140, Subproject No. 412, sponsored by the University of Minnesota, 1940.)

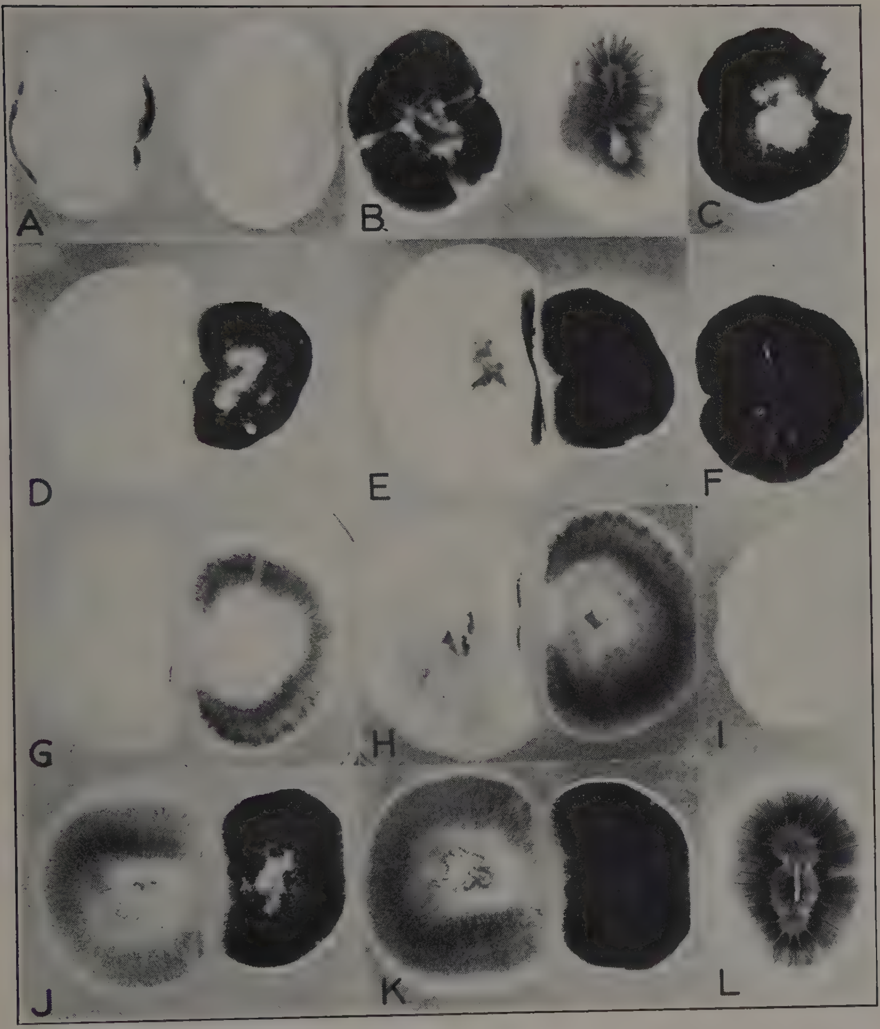

FIG. 2. Comparison of 15-day colonies of various saltants of *Verticillium albo-atrum* grown on Petri plates of potato-dextrose agar:

Upper, A, and lower, B, surface of parent type (dark) and intermediate (light) colonies.

Upper, C, and lower, F, surface of microsclerotial type with white sector.

Upper, D, and lower, E, surface of white (mycelial) and black (microsclerotial) types. Note difference in growth rate and apparent antagonism between the colonies. The production of microsclerotia in the mycelial colony adjacent to the black colony is of interest because the mycelial type when grown alone remains entirely white.

Upper, G, and lower, H, surface of white aerial type and intermediate submerged type. Note the carbonized hyphae in the submerged colony.

Upper, I, and lower, L, surface of intermediate type with aerial mycelium.

Upper, J, and lower, K, surface of intermediate submerged and black type of colony. Note contrast between black (microsclerotial) and submerged (carbonized hyphae) colonies.

advanced proof in 1854 that no real difference exists in the manner in which the spores of the two fungi are produced or in the manner in which they unite in heads. In 1879, Reinke and Berthold (12) isolated a fungus from potato plants affected by a disease referred to at that time as "Kräusel Krankheit." They accepted Hoffman's views and referred the organism they had just isolated to the genus *Verticillium,* calling it *V. albo-atrum.* In 1913 Klebahn (6) isolated *V. dahliae* from dahlia plants, and believed it sufficiently distinct from *V. albo-atrum* to entitle it to specific rank. Although, as van der Lek (7) has pointed out, the difference between the two organisms in culture is very slight, a number of authors have accepted Klebahn's work.

In his paper Klebahn admits that certain of the morphological and cultural characteristics by which he distinguished between his organism and *V. albo-atrum* are of little importance. The principal difference between them appears to be in the abundant production of true microsclerotia by *V. dahliae* and the complete absence of such structures in cultures of *V. albo-atrum.*

Referring to morphological changes in his cultures Rudolph (13) writes, "I have cultures of Verticillium which once produced dense masses of black, carbonized hyphae in culture and others which produced sclerotia in such quantity that these bodies formed a continuous, gritty crust on the surface of the medium. With continued cultivation on artificial media these strains have largely lost their ability to produce black mycelium and sclerotia. The colonies now consisting of cottony white mycelium with little or no darkening of the medium by black hyphae or sclerotia. In some cultures, spore production has been suppressed." Other workers have reported changes in morphology, supposedly brought about by continued cultivation on artificial media (Chaudhuri (3), van der Lek (7), Pethybridge (11). Also, Bewley (1) reports having isolated 6 strains of *V. albo-atrum* from plants suffering hadromycosis, in which the amount of sclerotia in culture varied, according to his standard, from 0 per cent to 100 per cent. From his inoculation studies with these strains he concluded that there was some evidence that virulence is distinctly associated with the amount of sclerotia or carbonized hyphae produced.

For the reasons just given it is not easy to accept the slight morphological differences ascribed to some of the so-called species of *Verticillium* as sufficient claim for specific rank for them. Moreover, the wide range of variability in the appearance, as well as in measurements, of the mycelium, conidiophores, conidia, etc. of the saltants the writer has obtained compels him to agree with Rudolph's (13) conclusions in regard to the taxonomy of the genus *Verticillium,* and to further suggest that most of the "strains" and "forms" of the genus are merely variants of the species *albo-atrum* and should be designated as such. For instance, Bewley's (1) 6 strains of *albo-atrum,* as based on microsclerotial production, have their counterpart in the cultures selected from sectors of a monosporic culture. All of the forms of van Beyma Thoe Kingma (2), based upon microsclerotial characters, appear to be represented in the array of cultures derived from a single spore. Should we accept the above cited descriptions that *V. albo-atrum* forms dark mycelial colonies, whereas *V. dahliae* forms abundant microsclerotia, we are still faced with the fact that both of these types are obtainable from a monosporic culture of *V. albo-atrum.*

The writer believes that the cultural changes in *Verticillium,* as reported by Chaudhuri, van der Lek, Pethybridge, and Rudolph, might easily have occurred as a result of sectoring and unconscious selection toward a "type" in successive transfers of the organism. Since the raised, white type of growth is most convenient to the transfer needle, it is not surprising that the subsequent cultures "lost their ability to produce black mycelium," and that "the colonies now consist of cottony white mycelium with little or no darkening of the medium by black hyphae."

The various cultures herein reported have retained their characteristics through many transfers and, with the exception of additional sectors that still occur, have remained remarkably constant in growth habits and in general appearance.

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LITERATURE CITED

1. BEWLEY, W. F. "Sleepy disease" or wilt of the tomato. Sixth Ann. Rept. Cheshunt Exp. and Res. Stat. pp. 26–38. 1920.
2. BEYMA THOE KINGMA, F. H. VAN. Ueber einige Formen von *Verticillium dahliae* Klebahn. Antonie van Leeuwenhoek **6**: 34–47. 1940.
3. CHAUDHURI, H. A study of the growth in culture of *Verticillium albo-atrum* R. et B. Ann. Bot. (London) **37**: 519–539. 1923.
4. CORDA, A. Icones fungorum **2**: 15. J. G. Calve (Prague). 1838.
5. HOFFMAN, H. Spermatien bei einem Fadenpilze. Bot. Zeit. **12**: 249–254, 265–269. 1854.
6. KLEBAHN, H. Beiträge zur Kenntnis der Fungi imperfecti. I. Eine Verticillium-Krankheit auf Dahlien. Mycol. Centralbl. **3**: 49–66. 1913.
7. LEK, H. A. A. VAN DER. Over de z. g. "Verwelkingsziekten" in het bijzonder die, welke door *Verticillium albo-atrum* veroorzaakt worden. Tijdschr. Plantenziekten. **24**: 205–219. 1918.
8. ————. Onderzoekingen over tracheomycosen: De verticilliose van den komkommer. Med. Landbouwhoogesch. (Wageningen) **15**: 1–45. 1918.
9. MEER, J. H. H. VAN DER. Verticillium-wilt of herbaceous and woody plants. Med. Landbouwhoogesch. (Wageningen) **28**: 1–82. 1925.
10. NEES VON ESENBECK, C. G. Das System der Pilze und Schwämme. Stahelschen Buchhandlung (Würzburg). 329 pp. 1816.
11. PETHYBRIDGE, G. H. The Verticillium disease of the potato. Sci. Proc. Roy. Dublin Soc. N. S. **15**: 93–92. 1916.
12. REINKE, J., and G. BERTHOLD. Die Zersetzung der Kartoffel durch Pilze. Untersuch. Bot. Lab. Univ. Göttingen **1**: 1–100. 1879. Wiegandt, Hempel, und Parey (Berlin).
13. RUDOLPH, B. A. Verticillium hadromycosis. Hilgardia **5**: 201–351. 1931.

PHYTOPATHOLOGICAL NOTES

Resistance to the Common Mosaic Disease of Tobacco.—During recent years two types of resistance to the tobacco-mosaic disease have been described. In 1933, Nolla and Roque[1] reported that the Ambalema variety of tobacco, obtained in Colombia, South America, was resistant to common mosaic. Since then many mosaic-resistant collections have been secured from the same area and a few, such as T. I. 448, were more resistant than Ambalema.[2, 3] This resistance, which can be conveniently referred to as the Ambalema type, is dependent on two recessive pairs of genes. In 1938, Holmes[4] reported on tobacco-mosaic resistance obtained by crossing *Nicotiana tabacum* with *N. glutinosa.* The glutinosa type of mosaic reaction was shown to depend on a single dominant pair of genes. Commentators in various countries—Brazil, Australia, England, and Russia—have generally taken the view that the glutinosa type of mosaic resistance was greatly preferable to the Ambalema type because it resulted in the complete elimination of the virus in the infected plant. Smith[5] and Bawden,[6] in recently discussing resistance to tobacco-mosaic disease, mention only the glutinosa type. These and other writers refer to this as a *local* necrotic lesion reaction. Holmes, however, very definitely described his mosaic resistant tobacco as having a ''necrotic type of response,'' which might remain localized in the older plants.

Breeding tobaccos resistant to mosaic is a matter of great importance, and the practical merits of Ambalema and glutinosa resistance can be decided only by actual field tests. Such tests were conducted in 1939 and 1940 (Fig. 1). Genotypes with the glutinosa resistance, when field-inoculated with the mosaic virus, developed systemic necrosis freely and rapidly. Many were completely killed, and some of the plants affected were even approaching maturity. It appears, therefore, that the typical field reaction is systemic necrosis and not local necrotic lesions. Such genotypes should logically be considered as highly susceptible, while ordinary tobacco is, by contrast, quite tolerant. Studies by one of the writers (McKinney) show that high temperatures favor necrosis in tobacco carrying glutinosa factors, and temperatures producing necrosis are common during the summer in the

[1] Nolla, J. A. B. and A. Roque. A variety of tobacco resistant to ordinary tobacco mosaic. Jour. Dept. Agr., P. R., 19: 301–303. 1933.

[2] Clayton, E. E., H. H. Smith, and H. H. Foster. Mosaic resistance in *Nicotiana tabacum* L. Phytopath. 28: 286–288. 1938.

[3] McKinney, H. H. Comparative studies on two genotypes of *Nicotiana tabacum* resistant to Nicotiana Virus.1 Phytopath. 29: 16. 1939.

[4] Holmes, F. O. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopath. 28: 553–560. 1938.

[5] Smith, K. M. The virus: life's enemy. 1940.

[6] Bawden, F. C. Problems in breeding for disease resistance. Chron. Botanica 6: 247. 1941.

major tobacco-producing areas of this country. Many of the glutinosa-tobacco hybrids, furthermore, are killed outright at a lower temperature, and over a wider plant age range, than is the case with the *N. glutinosa* parent. The widespread impression that the glutinosa reaction in tobacco is limited to the local lesion type is probably due to the fact that much of the work has been done under rather cool greenhouse conditions. It is certainly true that if a genotype could be developed with a completely localized necrotic reaction, this would be a satisfactory form of mosaic resistance. However, the prospects for achieving this do not appear promising. Therefore, under tobacco-producing conditions that prevail in this country, it

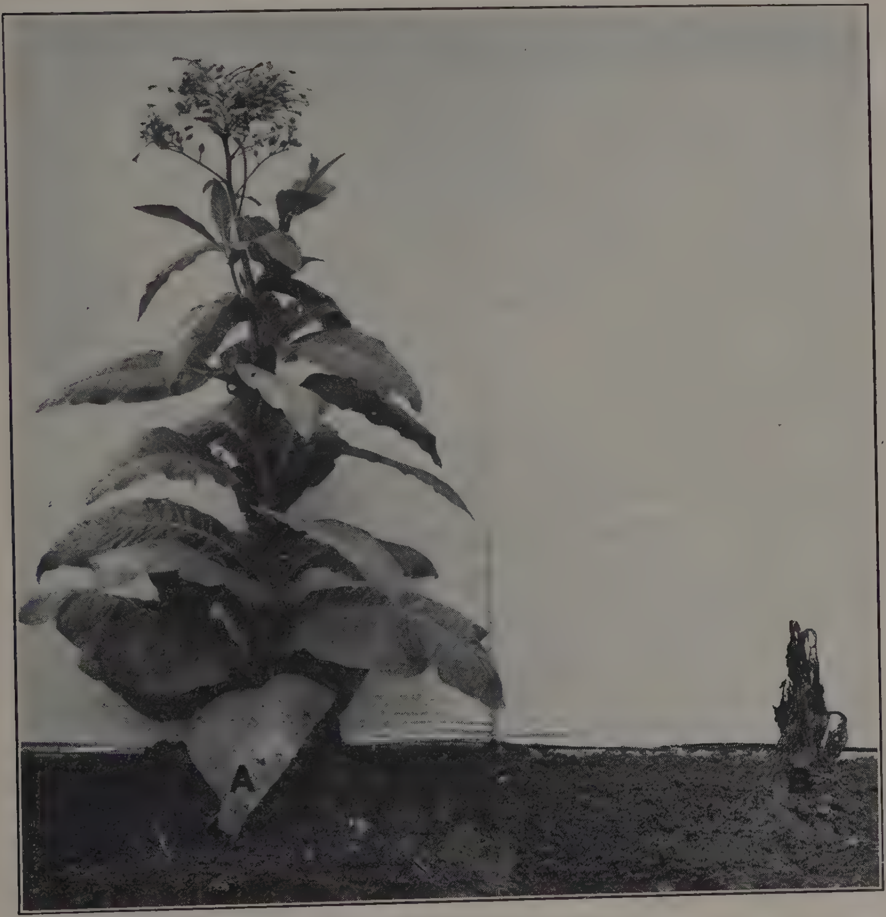

FIG. 1. Tobacco plants inoculated with common mosaic virus on July 23, 1940, a month before the picture was taken. The plant on the left (A) had the Ambalema type of mosaic resistance and the only result from the inoculation was a few diffuse spots on the basal leaves. The plant on the right (B) had the glutinosa type of mosaic reaction. Six days after inoculation it showed stem necrosis and soon was completely dead. The plants were of the same age and size, approximately 2 feet high, at the time of inoculation.

does not seem that the glutinosa type of mosaic resistance has any practical value.

The Ambalema type of mosaic resistance has been subjected to extensive field testing for 6 years. The resistance has been maintained through numerous backcrosses, and tobacco varieties possessing Ambalema resistance will certainly be introduced into cultivation in the near future. Furthermore, if an even higher degree of resistance is desired, genotypes such as T. I. 448 are available.—E. E. CLAYTON and H. H. MCKINNEY, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

A Preliminary Report on Genetic Studies on Pathogenicity and the Nature of Saltation in Venturia inaequalis.—Two classes of monoascosporic isolates were studied for pathogenicity to leaves of certain differential apple varieties in the greenhouse: (1) "lesion" (inciting typical, sporulating lesions) and (2) "fleck" (inciting yellowish flecks, usually without sporulation, sometimes with slight sporulation). Crosses were made *in vitro* and the 8 ascospores from an ascus of each progeny were cultured and their lines studied for pathogenicity. Lesion × lesion gave 8 lines lesion: fleck × fleck, 8 lines fleck: lesion × fleck, 4 lines lesions, 4 fleck.

Similar studies showed that certain sector lines are genetically different from the lines from which they sprang. Original monoascosporic lines D 5 (pathogenic) × D 8 (pathogenic) gave 8-spore asci: 8 pathogenic lines. D 5 × D 8 sector (non-pathogenic) gave 8-spore asci; 4 pathogenic and 4 non-pathogenic lines. D 5 × sector from D 8 sector (non-pathogenic) gave 8-spore asci: 2 pathogenic and 6 non-pathogenic lines. Certain crosses of non-pathogenic sector lines with pathogenic lines gave 4-spore asci, whose 4 lines showed the same pathogenic reactions as the pathogenic parental line. There is no known possibility that heterocaryosis initiated the sectors studied; they apparently arose by mutation.—G. W. KEITT and M. H. LANGFORD, University of Wisconsin, Madison, Wisconsin.